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## (54) Title: VACCINE CONSTRUCTS AND USES THEREOF AGAINST STAPHYLOCOCCUS INFECTIONS

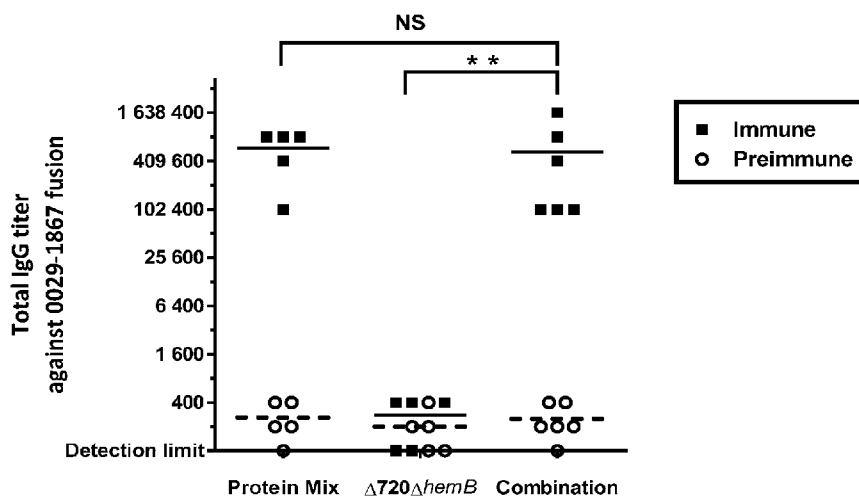


FIG. 16

(57) Abstract: There is provided a fusion construct of formula (I): X-A-linker-B-Z (I) wherein : (1) A and B are identical or different and are independently: (a) a polypeptide comprising a SACOL0029 polypeptide as set forth in any one of the sequences depicted in FIG. 24 (SEQ ID NOs: 5 and 121 to 131), a SACOL0264 polypeptide (SEQ ID NO : 185), a SACOL0442 polypeptide as set forth in any one of the sequences depicted in FIG. 22D (SEQ ID NOs: 29 and 82 to 92), a SACOL0718 polypeptide (SEQ ID NO : 186), a SACOL0720 polypeptide as set forth in any one of the sequences depicted in FIGs. 23I-J (SEQ ID NOs: 11 and 109 to 120), a SACOL1353 polypeptide (SEQ ID NO : 187), a SACOL1416 polypeptide (SEQ ID NO : 188), a SACOL1611 polypeptide (SEQ ID NO : 189), a SACOL1867 polypeptide as set forth in any one of the sequences depicted in FIG. 25D (SEQ ID NOs: 152 to 164), a SACOL1912 polypeptide (SEQ ID NO : 43), a SACOL1944 polypeptide (SEQ ID NO : 190), a SACOL2144 polypeptide (SEQ ID NO : 191), a SACOL2365 polypeptide (SEQ ID NO : 192), a SACOL2385 polypeptide (SEQ ID NO : 50) or a SACOL2599 polypeptide (SEQ ID NO : 193), based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in



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NCBI Reference Sequence NC\_002951.2; (b) a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a); (c) a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a) or (b); (d) a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a) to (c); or (e) a polypeptide comprising an immunogenic variant comprising at least 13 consecutive amino acids of any one of (a) to (c); (2) the linker is an amino acid sequence of at least one amino acid or is absent; (3) X is absent or is an amino acid sequence of at least one amino acid; and (4) Z is absent or is an amino acid sequence of at least one amino acid. Also provided are compositions and kits comprising the fusion and uses of these fusions, compositions and kits.

VACCINE CONSTRUCTS AND USES THEREOF AGAINST *STAPHYLOCOCCUS* INFECTIONS

## CROSS REFERENCE TO RELATED APPLICATIONS

[0001] N.A.

## STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] N.A.

## FIELD OF THE INVENTION

[0003] The present invention relates to vaccine constructs and uses thereof against *Staphylococcus* infections. More specifically, the present invention is concerned with vaccine constructs combining antigens and their uses against *Staphylococcus* infections such as bovine intramammary infections (IMI).

## REFERENCE TO SEQUENCE LISTING

[0004] Pursuant to 37 C.F.R. 1.821(c), a sequence listing is submitted herewith as an ASCII compliant text file named SEQUENCE LISTING USP62411120\_ST25, created on October 5, 2017 and having a size of 278 kilobytes. The content of the aforementioned file is hereby incorporated by reference in its entirety.

## BACKGROUND OF THE INVENTION

[0005] Bovine mastitis is the most frequent and costly disease for dairy producers and *Staphylococcus aureus* is considered to be the transmittable bacterium that is the most often responsible for the development of the disease (Sears *et al.*, 2003). Staphylococcal IMIs, which may lead to mastitis, are difficult to treat and frequent relapses are common (Sandholm *et al.*, 1990).

[0006] The development of vaccines for the prevention and control of *S. aureus* IMIs has been extensively investigated although no formulation has demonstrated protective efficacy to date. This is probably because of inadequate vaccine targets (Middleton, 2008; Middleton 2009), high diversity among strains capable of provoking mastitis (Buzzola, 2007; Kerro-Dego, 2006; Middleton, 2008) or the failure to elicit an appropriate immune response (Bharathan, 2011; Ferens, 2000; Fowler, 2014; Proctor, 2012). It is increasingly understood that immunity solely based on vaccine-induced antibodies may be important but is however insufficient for inducing protection against *S. aureus* (Middleton 2008; Middleton 2009). It appears that cell mediated immunity (CMI) based on Th1 and Th17 type responses may be necessary to complete the protection (Fowler, 2014; Lin, 2009; Proctor, 2012; Spellberg, 2012).

[0007] Bacterial susceptibility to antibiotics *in vitro* is a poor predictor of therapeutic efficacy in chronically infected cows (Owens *et al.*, 1997). Although infections that follow treatment of mastitis can be due to newly acquired strains, they are often the result of the persistence of the original infective organism (Sandholm *et al.*, 1990; Myllys *et al.*, 1997). Existing therapies thus often fail to eliminate the infection and it would be highly desirable to find novel approaches to prevent or treat staphylococcal IMI.

[0008] A lack of vaccine efficacy and protective ability has been noted for commercially available *S. aureus* vaccines (Middleton, 2008). Thus, it would be highly desirable to use highly efficient *S. aureus* antigens that are

known to be expressed during IMI as vaccine components for protection against IMI and mastitis.

**[0009]** The present invention seeks to meet these and other needs.

**[0010]** The present description refers to a number of documents, the content of which is herein incorporated by reference in their entirety.

#### SUMMARY OF THE INVENTION

**[0011]** In a first aspect, the present invention provides fusion polypeptides displaying increased immunogenicity and their use as vaccine against staphylococcal IMI.

**[0012]** A characteristic of staphylococcal such as *S. aureus* IMI is the ability of *S. aureus* to persist within host cells. In particular, *S. aureus* small colony variants (SCVs) do not generally generate invasive infections and can be internalized in host cells. In a further aspect therefore, the present invention provides live-attenuated *S. aureus* strains for vaccine purposes based on the phenotypic aspects of SCVs to provide an immune response against such strains and increase the vaccine protective efficacy.

**[0013]** In an aspect, the present invention provides the following items:

**[0014]** Item 1: A fusion construct of formula (I): X-A-linker-B-Z (I) wherein: (1) A and B are identical or different and are independently: (a) a polypeptide comprising a SACOL0029 polypeptide as set forth in any one of the sequences depicted in FIG. 24 (SEQ ID NOs: 5 and 121 to 131), a SACOL0264 polypeptide (SEQ ID NO : 185), a SACOL0442 polypeptide as set forth in any one of the sequences depicted in FIG. 22D (SEQ ID NOs: 29 and 82 to 92), a SACOL0718 polypeptide (SEQ ID NO : 186), a SACOL0720 polypeptide as set forth in any one of the sequences depicted in FIGs. 23I-K (SEQ ID NOs: 11 and 109 to 120), a SACOL1353 polypeptide (SEQ ID NO : 187), a SACOL1416 polypeptide (SEQ ID NO : 188), a SACOL1611 polypeptide (SEQ ID NO : 189), a SACOL1867 polypeptide as set forth in any one of the sequences depicted in FIG. 25D (SEQ ID NOs: 152 to 164), a SACOL1912 polypeptide (SEQ ID NO : 43), a SACOL1944 polypeptide (SEQ ID NO : 190), a SACOL2144 polypeptide (SEQ ID NO : 191), a SACOL2365 polypeptide (SEQ ID NO : 192), a SACOL2385 polypeptide (SEQ ID NO : 50) or a SACOL2599 polypeptide (SEQ ID NO : 193), based on the gene nomenclature from the *Staphylococcus aureus* COL (SACOL) genome set forth in NCBI Reference Sequence NC\_002951.2; (b) a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a); (c) a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a) or (b); (d) a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a) to (c); or (e) a polypeptide comprising an immunogenic variant comprising at least 13 consecutive amino acids of any one of (a) to (c); (2) the linker is an amino acid sequence of at least one amino acid or is absent; (3) X is absent or is an amino acid sequence of at least one amino acid; and (4) Z is absent or is an amino acid sequence of at least one amino acid.

**[0015]** Item 2: The construct of item 1, wherein (1) (a) is a polypeptide comprising a SACOL0029 polypeptide as set forth in any one of the sequences depicted in FIG. 24 (SEQ ID NOs: 5 and 121 to 131), a SACOL0442 polypeptide as set forth in any one of the sequences depicted in FIG. 22D (SEQ ID NOs: 29 and 82 to 92), a

SACOL0720 polypeptide as set forth in any one of the sequences depicted in FIGs. 23I-K (SEQ ID NOs: 11 and 109 to 120), or a SACOL1867 polypeptide as set forth in any one of the sequences depicted in FIG. 25D (SEQ ID NOs: 152 to 164).

**[0016]** Item 3: The construct of item 2, wherein at least one of A and B is (a) a polypeptide comprising a SACOL0029 polypeptide as set forth in any one of the sequences depicted in FIG. 24 (SEQ ID NOs: 5 and 121 to 131); (b) a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a); (c) a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a) or (b); (d) a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a) to (c); or (e) a polypeptide comprising an immunogenic variant comprising at least 13 consecutive amino acids of any one of (a) to (d); and the other one of A and B is (a') a polypeptide comprising a SACOL1867 polypeptide as set forth in any one of the sequences depicted in FIG. 25D (SEQ ID NOs: 152 to 164); (b') a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a'); (c') a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a') or (b'); (d') a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a') to (c'); or (e') a polypeptide comprising an immunogenic variant comprising at least 12 consecutive amino acids of any one of (a') to (d').

**[0017]** Item 4: The construct of item 2, wherein at least one of A and B is (a) a polypeptide comprising a SACOL0442 polypeptide as set forth in any one of the sequences depicted in FIG. 22D (SEQ ID NOs: 29 and 82 to 92); (b) a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a); (c) a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a) or (b); (d) a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a) to (c); or (e) a polypeptide comprising an immunogenic variant comprising at least 13 consecutive amino acids of any one of (a) to (d); and the other one of A and B is (a') a polypeptide comprising a SACOL0720 polypeptide as set forth in any one of the sequences depicted in FIGs. 23I-K (SEQ ID NOs: 11 and 109 to 120); (b') a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a'); (c') a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a') or (b'); (d') a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a') to (c'); or (e') a polypeptide comprising an immunogenic variant comprising at least 12 consecutive amino acids of any one of (a') to (d').

**[0018]** Item 5: The construct of item 2, wherein A and B are identical or different and are (a) a polypeptide comprising a SACOL0720 polypeptide as set forth in any one of the sequences depicted in FIGs. 23I-K (SEQ ID NOs: 11 and 109 to 120); (b) a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a); (c) a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a) or (b); (d) a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a) to (c); or (e) a polypeptide comprising an immunogenic variant comprising at least 13 consecutive amino acids of any one of (a) to (d).

**[0019]** Item 6: The construct of any one of items 1-2 and 4, wherein said immunogenic fragment (d) comprises

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one or more of the following amino acid sequences: KDTINGKSNKSRNW (SEQ ID NO: 34); and KDGGKYTLESHKELQ (SEQ ID NO: 1).

**[0020]** Item 7: The construct of item 6, wherein said immunogenic fragment (d) comprises one or more of the following amino acid sequences : STQNSSSVQDKQLQKVEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIHLEGTYTVAG RVYTPKRNITLNKEVTLKELDHIIRFAHISYGLYMGEHLPGKNIVINTKDGGKYTLESHKELQKDRENVKINTADIK NVTFKLKSVNDIEQV (SEQ ID NO: 30); DKQLQKVEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIHLEGTYTVAGRVYTPKRNIT LNKEVTLKELDHIIRFAHISYGLYMGEHLPGKNIVINTK (SEQ ID NO :32); and DKQLQKVEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIHLEGTYTVAGRVYTPKRNIT LNKEVTLKELDHIIRFAHISYGLYMGEHLPGKNIVINTKDGGKYTLESHKELQKDRENVKINTADIKNVTFKLKSV NDIEQV (SEQ ID NO : 33).

**[0021]** Item 8: The construct of any one of items 1-2 and 4-5, wherein said immunogenic fragment (d) comprises one or more of the following amino acid sequences: QFGFDLKHKKDALA (SEQ ID NO: 21); TIKDQQKANQLAS (SEQ ID NO: 22); KDINKIYFMTDVL (SEQ ID NO: 23); and DVDLGGPTFVLND (SEQ ID NO: 24).

**[0022]** Item 9: The construct of item 8, wherein said immunogenic fragment (d) comprises one or more of the following amino acid sequences: RASLSSEIKYTAPHDVTIKDQQKANQLASELNNQKIPHFYNYKEVIHTKLYKDNLFDVKAKEPYNVTITSDKYIPNTD LKRGQADLFVAEGSIKDLVKHKKHKGKAIIGTKKHHVNIKLKRDINKIYFMTDVLGGPTFVLNDKDYQEIRKYTKAK HIVSQFGFDLKHKKDALALEKAKNKVDKSIETRSEAISSISLTG (SEQ ID NO : 12); ASLSSEIKYTAPHDVTIKDQQKANQLASELNNQKIPHFYNYKEVIHTKLYKDNLFDVKAKEPYNVTITSDKYIPNTDL KRGQADLFVAEGSIKDLVKHKKHKGKAIIGTKKHHVNIKLKRDINKIYFMTDVLGGPTFVLNDKDYQEIRKYTKAKHI VSQFGFDLKHKKDALALEKAKNKVDKSIETRSEAISSISLTG (SEQ ID NO: 13); ASLSSEIKYTAPHDVTIKDQQKANQLASELNNQKIPHFYNYKEVIHTKLYKDNLFDVKAKEPYNVTITSDKYIPNTDL KRGQADLFVAEGSIKDLVKHKKHKGKAIIGTKKHHVNIKLKRDINKIYFMTDVLGGPTFVLNDKDYQE (SEQ ID NO: 14); KDINKIYFMTDVLGGPTFVLNDKDYQEIRKYTKAKHIVSQFGFDLKHKKDALA (SEQ ID NO : 15); KDINKIYFMTDVLGGPTFVLNDKD (SEQ ID NO : 16); KDINKIYFMTDVLGGPTFVLNDKDY (SEQ ID NO : 17); KDINKIYFMTDVLGGPTFVLND (SEQ ID NO : 19); SQFGFDLKHKKDALA (SEQ ID NO: 18); and KHIVSQFGFDLKHKKDALA (SEQ ID NO: 20).

**[0023]** Item 10: The construct of any one of items 1-3, wherein said immunogenic fragment (c') comprises one or more of the following amino acid sequences: PYNGVVSFKDATGF (SEQ ID NO: 165); AHPNGDKGNNGGIYK (SEQ ID NO: 167); SISDYPGDEDISVM (SEQ ID NO: 169); RGPKGFNFNENVQA (SEQ ID NO: 172); QFESTGTIKRIKDN (SEQ ID NO: 175); and GNSGSPVLNSNNEV (SEQ ID NO: 178).

**[0024]** Item 11: The construct of item 10, wherein said immunogenic fragment (d) comprises the following amino acid sequence:

TQVKDTNIFPYNGVVSFKDATGFVIGKNTIITNKHVSKDYKVGDRITAHPNGDKGNNGGIYKIKSISDYPGDEDISVM  
NIEEQAVERGPKGFNFNENVQAFNFAKDAKVDDKIKVIGYPLPAQNSFKQFESTGTIKRIKDNLNFDAYIEPGNSG  
SPVLNSNNEVIGVYGGIGKIGSEYNGAVYFTPQIKDFIQKHIEQ (SEQ ID NO: 39).

**[0025]** Item 12: The construct of any one of items 1 to 11, wherein the linker comprises at least four identical or different amino acids selected from the group consisting of glycine, serine, alanine, aspartate, glutamate and lysine.

**[0026]** Item 13: The construct of any one of items 1 to 12, wherein the linker comprises (GGGGS)<sub>n</sub> (SEQ ID NO: 67), (ERKYK)<sub>n</sub> (SEQ ID NO: 61); or (EAAAK)<sub>n</sub> (SEQ ID NO: 63), wherein n=1 to 5.

**[0027]** Item 14: The construct of any one of items 1 to 13, wherein said X comprises a polyhistidine of 6 to 10 amino acids.

**[0028]** Item 15: The construct of any one of items 1 to 13, wherein said X is absent.

**[0029]** Item 16: The construct of any one of items 1 to 15, wherein said Z is absent.

**[0030]** Item 17: An isolated nucleic acid molecule encoding the construct defined in any one of items 1 to 16.

**[0031]** Item 18: A vector comprising the isolated nucleic acid defined in item 17.

**[0032]** Item 19: A host cell comprising the vector defined in item 18.

**[0033]** Item 20: The cell of item 19, which is a live attenuated form of *Staphylococcus aureus*.

**[0034]** Item 21: The cell of item 20, wherein the live attenuated form of *Staphylococcus aureus* has a stabilized small colony variant (SCV) phenotype.

**[0035]** Item 22: The cell of item 21, wherein the live attenuated form of *Staphylococcus aureus* having a stabilized SCV phenotype is a  $\Delta hemB\Delta 720$  *S. aureus*.

**[0036]** Item 23: A composition comprising: (A) at least one of the constructs defined in any one of items 1 to 16; at least one of the nucleic acid molecules defined in item 17; at least one of the vectors defined in item 18; or at least one of the cells defined in any one of items 19 to 22; and (B) (i) the polypeptide defined in any one of items 1 to 11; (ii) a live attenuated *Staphylococcus aureus*; (iii) a pharmaceutically acceptable excipient; (iv) an adjuvant; or (v) a combination of at least two of (i) to (iv).

**[0037]** Item 24: The composition of item 23, wherein the live attenuated form of *Staphylococcus aureus* expresses: (a) a polypeptide comprising a SACOL0029 polypeptide as set forth in any one of the sequences depicted in FIG. 24 (SEQ ID NOs: 5 and 121 to 131), a SACOL0264 polypeptide (SEQ ID NO : 185), a SACOL0442 polypeptide as set forth in any one of the sequences depicted in FIG. 22D (SEQ ID NOs: 29 and 82 to 92), a SACOL0718 polypeptide (SEQ ID NO : 186), a SACOL0720 polypeptide as set forth in any one of the sequences depicted in FIGs. 23I-K (SEQ ID NOs: 11 and 109 to 120), a SACOL1353 polypeptide (SEQ ID NO : 187), a SACOL1416 polypeptide (SEQ ID NO : 188), SACOL1611 (SEQ ID NO : 189), a SACOL1867 polypeptide as set forth in any one of the sequences depicted in FIG. 25D (SEQ ID NOs: 152 to 164), a SACOL1912 polypeptide (SEQ ID NO : 43), SACOL1944 (SEQ ID NO : 190), a SACOL2144 polypeptide (SEQ ID NO : 191), a

SACOL2365 polypeptide (SEQ ID NO : 192), a SACOL2385 polypeptide (SEQ ID NO : 50) or a SACOL2599 polypeptide (SEQ ID NO : 193) based on the gene nomenclature from the *Staphylococcus aureus* COL (SACOL) genome set forth in NCBI Reference Sequence NC\_002951.2; (b) a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a); (c) a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a) or (b); (d) a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a) to (c); or (e) a polypeptide comprising an immunogenic variant comprising at least 13 consecutive amino acids of any one of (a) to (c).

**[0038]** Item 25: The composition of item 23 or 24, wherein the live attenuated form of *Staphylococcus aureus* has a stabilized small colony variant (SCV) phenotype.

**[0039]** Item 26: The composition of any one of items 23 to 25, wherein the adjuvant comprises alum, an oil (e.g., emulsified oil, mineral oil), saponin (e.g., Quil-A™), cyclic-diguanosine-5'-monophosphate (c-di-GMP), polyphosphasine, indolicidin, pathogen-associated molecular patterns (PAMPS) or a combination of at least two thereof.

**[0040]** Item 27: A method for preventing and/or treating a Staphylococcal intramammary infection (IMI) in a mammal, said method comprising administering to said mammal an effective amount of the construct defined in any one of items 1 to 16; of the nucleic acid molecule defined in item 17; of the vector defined in item 18; of the cell defined in any one of items 19 to 22; or of the composition defined in any one of items 23 to 26.

**[0041]** Item 28: The method of item 27, wherein said Staphylococcal IMI is caused by one or more *Staphylococcus aureus* strains.

**[0042]** Item 29: The method of item 27 or 28, wherein said mammal is a cow.

**[0043]** Item 30: A use of an effective amount of (i) the construct defined in any one of items 1 to 16; (ii) the nucleic acid molecule defined in item 17; (iii) the vector defined in item 18; of the cell defined in any one of items 19 to 22; (iv) the composition defined in any one of items 23 to 26; or (v) a combination of at least two of (i) to (iv), for preventing and/or treating a Staphylococcal intramammary infection (IMI) in a mammal.

**[0044]** Item 31: The use of item 30, wherein said Staphylococcal IMI is caused by one or more *Staphylococcus aureus* strains.

**[0045]** Item 32: The use of item 30 or 31, wherein said mammal is a cow.

**[0046]** Item 33: The construct defined in any one of items 1 to 16; the nucleic acid molecule defined in item 17; the vector defined in item 18; the cell defined in any one of items 19 to 22; the composition defined in any one of items 23 to 26 or a combination of at least two thereof, for use in the prevention and/or treatment of a Staphylococcal intramammary infection (IMI) in a mammal.

**[0047]** Item 34: The construct, nucleic acid molecule, vector, cell, composition or combination of item 33, wherein said Staphylococcal IMI is caused by one or more *Staphylococcus aureus* strains.

**[0048]** Item 35: The construct, nucleic acid molecule, vector, cell or composition of item 33 or 34, wherein said

mammal is a cow.

**[0049]** Item 36: A kit for preventing and/or treating a Staphylococcal intramammary infection (IMI) in a mammal comprising: (A) (i) at least one of the constructs defined in any one of items 1 to 16; (ii) at least one of the nucleic acid molecules defined in item 17; (iii) at least one of the vectors defined in items 18; (iv) at least one of the cells defined in any one of items 19 to 22; or (v) a combination of at least two of (i) to (iv), and (B) (i) the polypeptide defined in any one of items 1 to 11; (ii) a live attenuated *Staphylococcus aureus*; (iii) a pharmaceutically acceptable excipient; (iv) an adjuvant; (v) instructions for using the kit for preventing and/or treating a Staphylococcal intramammary infection (IMI) in a mammal; or (vi) a combination of at least two of (i) to (v).

#### BRIEF DESCRIPTION OF THE DRAWINGS

**[0050]** In the appended drawings :

**[0051]** **FIGs. 1A-D.** shows serum total IgG (FIG. 1A), IgG1 (FIG. 1B), and IgG2 (FIG. 1C) titers for the vaccinated (9) and placebo (10) cows for each antigen of the vaccine, namely SACOL0029, SACOL0442, SACOL0720, SACOL1867, SACOL1912, and SACOL2385, four weeks after the second immunization (just before the experimental infection). In FIG. 1D, the IgG2/IgG1 ratio is represented for the vaccinated cows. In FIGs. 1A, B and C, open circles (○) represent data for the vaccinated cows, black squares (■) represent data for the placebo cows. Each symbol represents the titer for one cow. Horizontal lines represent the medians: dashed lines represent the medians for the vaccinated cows while continuous lines represent the medians for the placebo cows. In FIGs. 1A, B and C, titers for the vaccinated cows are higher than the titers for the placebo cows ( $P < 0.0001$ ). In FIG. 1D, symbols represent the ratio IgG2/IgG1 for each cow. Horizontal lines represent the medians. The different letters show statistical differences. \*\*\*,  $P < 0.001$ .

**[0052]** **FIG. 2.** shows antigen dependent proliferation of blood CD4+ cells from the vaccinated cows (9) and placebo cows (10) four weeks after the second immunization for each antigen. Each symbol represents the percentage of CD4+ cells that have proliferated for each cow after a week of incubation with the positive control (ConA) or each antigen, namely SACOL0029, SACOL0442, SACOL0720, SACOL1867, SACOL1912, and SACOL2385. Open circles (○) represent data for the vaccinated cows, black squares (■) represent data for the placebo cows. Horizontal lines represent the medians: dashed lines represent the medians for the vaccinated cows while continuous lines represent the medians for the placebo cows. Statistical analysis: Mixed procedure of SAS. The symbol \* shows the statistical differences between the vaccinated and the placebo groups for antigens SACOL0029, SACOL0442, SACOL0720 and SACOL1912 (\*,  $P < 0.05$ ). In addition, the proliferation of CD8+ cells was similar for the vaccinated and placebo cows for all antigens with the exception of the antigen SACOL0720 for which higher proliferation of the CD8+ cells was observed for the vaccinated cows (data not shown).

**[0053]** **FIG. 3.** shows experimental *S. aureus* intramammary infections in dairy cows. Four weeks and 4 days after the second immunization, 63 Colony Forming Unit (CFU) of *S. aureus* were infused into 3 of the 4 quarters of the vaccinated (9) and placebo cows (10) at the evening milking (day 1, arrow in **FIG. 3**). Aseptic milk samples were taken at morning milking and Somatic Cell Counts (SCC) were determined by Valacta (Ste-Anne-

de-Bellevue, QC). Open circles (○) and the dashed line represent data for the vaccinated cows, while the black squares (■) and the continuous line represent data for the placebo cows. Each open circle represents the mean of SCC for all the infected quarters of the vaccinated cows (27) while each square represents the mean of SCC for all the infected quarters of the placebo cows (30 quarters). Over the challenge period, somatic cell counts in milk were found to be significantly lower for the vaccinated cows than for the placebo cows (\*\*\*;  $P < 0.001$ ).

**[0054] FIGs. 4A-C.** FIG. 4A shows the correlation between CFU and the SCC for each cow, and FIG. 4B shows the correlation between serum IgG1 titer against SACOL0442 and SCC for each cow. Each symbol represents data for one cow. In FIG. 4A, it represents the mean of SCC and CFU for the 3 infected quarters from the beginning to the end of the infection. In FIG. 4B, it represents the mean of SCC for the 3 infected quarters from the beginning to the end of the infection and the serum IgG1 titer against SACOL0442 four weeks after the second immunization and just before the experimental infection. Open circles represent data for the vaccinated cows and black squares represent data for the placebo cows. There is a strong correlation between the SCC/ml and the CFU/ml and a negative correlation between the SCC and the IgG1 titer against SACOL0442. In FIG. 4C, the correlation between SCC or CFU relative to milk IgG2 titer against SACOL0029 is shown for each cow ten days after experimental infection (6 weeks after the second immunization). Each symbol represents data for one cow ten days after the experimental infection. Aseptic milk samples were taken at morning milking and the viable counts of *S. aureus* were determined by 10-fold dilutions and plating on tryptic soy agar (TSA) plates while SCC were determined by Valacta (Ste-Anne-de-Bellevue, QC). SCC and CFU data for each cow is the mean of the data for the 3 infected quarters ten days after the experimental infection. Milk samples for the determination of milk IgG2 titers are the mix of an equivalent volume of milk from the 4 quarters of each cow 10 days after the experimental infection (6 weeks after the second immunization). Black squares (■) represent data for the placebo cows, open circles (○) represent data for the vaccinated cows.

**[0055] FIG. 5.** shows serum total IgG titers for the vaccinated cows for each antigen of a vaccine comprising the fused antigens SACOL0029 and SACOL1867 (shown as SACOL0029-1867) and the antigens SACOL0442 and SACOL0720. In the ELISA, the targeted antigens were SACOL0029-1867, SACOL0029, SACOL1867, SACOL0442 and SACOL0720. Each open circle represents the titer four weeks after the second immunization for each of the 11 cows whereas each black diamond represents the preimmune titer. Horizontal lines represent the medians: solid line for the preimmune serums, dotted line for the samples taken four weeks after immunization. Titers for the vaccinated cows are higher than the titers of the preimmune serums (\*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$  for the other antigens tested).

**[0056] FIG. 6** shows serum total IgG titers against the SACOL1867 antigen of mice immunised with the fusion protein (SACOL0029-1867; fusion), a combination of the separate proteins (SACOL0029 + SACOL1867; combination), the SACOL0029 protein only (0029) or SACOL1867 protein only (1867), in equivalent molar quantities. Open circles (○) represent data for preimmune titers, black squares (■) represent data for the immune titers. For the preimmune titers, preimmune sera were mixed equally between the 5 mice of each immunization group to obtain a preimmune pool titer, represented by one open circle per group. For the immune titers, each

square symbol represents the titer for one mouse. Horizontal lines represent the medians: black lines represent the medians for the immune serums while dashed lines (and the open circle) represent the medians for the preimmune serums pool. Titers for the vaccinated mice in the fusion, combination and 1867 groups are higher than the titers for the preimmune mice ( $P < 0.001$ ), and the titers of the mice that received SACOL0029 monovalent antigen only were not found to be significantly different from the titers of the preimmune pool against SACOL1867. Statistical significance between immune titers of fusion group versus the combination and the two monovalent vaccines groups is shown (\*\*\*:  $P < 0.001$ ).

**[0057] FIG. 7.** Serum total IgG (as measured by O.D. 450 nm in the ELISA assay) directed against a B-cell epitope sequence **KDGGKYTLESHKELQ** (SEQ ID NO: 1) contained in a fragment of the amino acid sequence of SACOL0442 (GEHLPKGNIVINT**KDGGKYTLESHKELQ**KDRENVKINTAD, SEQ ID NO: 2), and obtained from mice immunized with either a fusion of peptides encoded from SACOL0442 and SACOL0720 **KDGGKYTLESHKELQEAAAKEAAAKDINKIYFMTDVLGGPTFVLND** (SEQ ID NO: 3) (Group 1) or the peptide **KDGGKYTLESHKELQ** (SEQ ID NO: 1) encoded from SACOL0442 (Group 2). Each group was composed of 4 animals ( $n = 4$ ) that were injected two times with equimolar amounts of the sequence **KDGGKYTLESHKELQ** (SEQ ID NO: 1) (corresponding to 100  $\mu$ g of **KDGGKYTLESHKELQEAAAKEAAAKDINKIYFMTDVLGGPTFVLND** (SEQ ID NO: 3) for Group 1 and 31.25  $\mu$ g of **KDGGKYTLESHKELQ** (SEQ ID NO: 1) for Group 2) at a 2-week interval, and sera were prepared from blood harvested one week after the last injection. The ELISA assay was carried out with serum samples diluted 100 000 times and a fragment from SACOL0442 (GEHLPKGNIVINT**KDGGKYTLESHKELQ**KDRENVKINTAD, (SEQ ID NO: 2)) was used as the target antigen containing the peptide epitope **KDGGKYTLESHKELQ** (SEQ ID NO: 1). Individual data are expressed as circles on the graph and the medians by bars. The difference between groups was found statistically significant ( $P < 0.0286$ , Kuskal-Wallis test, GraphPad Prism™ 7.00).

**[0058] FIGs. 8A-B.** Deletion of *hemB* in ATCC29213 and  $\Delta 720$  strains of *Staphylococcus aureus*. (FIG. 8A) The *hemB* gene in the wild-type (WT) strain ATCC29213 and its isogenic mutant  $\Delta 720$  was deleted by homologous recombination and replacement with an *ermA* cassette to create the mutant strains  $\Delta hemB$  and  $\Delta 720\Delta hemB$ , respectively. Thick lines and numbers denote the PCR-amplified regions depicted in B for parental (1) and *hemB* deleted (2) strains. (FIG. 8B) PCR products of the WT strain and its isogenic  $\Delta hemB$  mutant (similar results were obtained with  $\Delta 720$  and  $\Delta 720\Delta hemB$  strains).

**[0059] FIGs. 9A-C.** show influence of *S. aureus*  $\Delta hemB$ ,  $\Delta 720$ , and  $\Delta hemB\Delta 720$  mutations on MAC-T cell infectivity. MAC-T cells were infected with each of the four strains for 3h, then were incubated with lysostaphin an additional 30 min ( $t=3h$ ), 12h or 24h and lysed for measurement of viable intracellular bacteria (CFU). (FIG. 9A) Relative recovery of the initial inoculum found within cells at  $t$  3h for  $\Delta 720$ , and (FIG. 9B) for  $\Delta hemB\Delta 720$  mutants. Results are normalized according to that obtained for ATCC 29213 (WT) or  $\Delta hemB$ , respectively, and are expressed as means with SD (\*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.001$ ; unpaired  $t$  test). (FIG. 9C) Means and SD of intracellular CFUs for WT and mutants at 12h (left) and 24h (right). A two-way ANOVA and Tukey's multiple comparisons test was used (\*:  $P \leq 0.05$ ; \*\*\*:  $P \leq 0.001$ ). All values indicate the mean of three independent experiments, each performed in triplicate.

**[0060] FIG. 10.** shows persistence of *S. aureus* ATCC 29213 (WT) and isogenic mutants within MAC-T cells over time. MAC-T cells were infected with each of the four strains for 3h, then were incubated with lysostaphin an additional 30 min, 12h or 24h and lysed for measurement of intracellular bacteria (CFU). Intracellular bacterial CFUs are expressed as the percentage of the initial inoculum after being transformed in base 10 logarithmic values (Log<sub>10</sub> CFU/ml). Values indicate the mean of three independent triplicate experiments with standard deviations.

**[0061] FIGs. 11A-B.** shows viability of MAC-T cells infected by *S. aureus* ATCC 29213 (WT) and isogenic mutants. MAC-T cells were infected with each of the four strains for 3h, then were incubated with lysostaphin for 12 h (FIG. 11A) or 24 h (FIG. 11B). MTT viability assays were then performed with a method described in Kubica et al., 2008. The results are reported as percent viability relative to uninfected cells and are expressed as the mean with SD of three independent experiments done in triplicate. Statistical significance with (Φ) symbol are compared to the WT (Two-way ANOVA and Tukey's multiple comparisons test: \* or Φ:  $P \leq 0.05$ ; \*\*:  $P \leq 0.01$ ; \*\*\*:  $P \leq 0.001$ ; ΦΦΦΦ:  $P \leq 0.0001$ ).

**[0062] FIG. 12.** Shows murine IMIs with the parental (WT) and  $\Delta hemB\Delta 720$  ( $\Delta\Delta$ ) strains. Mice were infected as previously described and glands harvested at the indicated hour (h) or day (D) after infection. Each column represents the median value of bacterial CFU counts for a group of glands, and ranges are indicated by bars. A minimum of six glands per group were used excepted for the WT strain at D7 (2 glands: only one mouse survived). Mortality of mice at specific time points is indicated by arrows. The asterisk indicates the clearance of  $\Delta hemB\Delta 720$  from glands (below the detection limit of 10 CFU/gland).

**[0063] FIG. 13.** Double mutant ( $\Delta 720\Delta hemB$ ) stimulates neutrophil influx in mammary glands to similar levels compared to WT in the first 24 hours following infection. Mice were infected as described in materials and methods, and a control group (PBS) of mice received a sterile PBS injection. Glands were harvested at indicated times, homogenized and kinetically assayed for MPO activity as described in materials and methods. Each dot represents MPO Units for one gland, which is shown as raw values adjusted by gram of gland. Means are represented by thick lines.

**[0064] FIG. 14.** Visual Inflammation of the large R4 and L4 mammary glands 24 h after mouse IMI with *S. aureus* ATCC 29213 (WT) and the double mutant  $\Delta 720\Delta hemB$  ( $\Delta\Delta$ ). Mice were infected as described in materials and methods, and control group (PBS) mice received a sterile PBS injection. Pictures show glands that were harvested after 24 h. In each panel, the R4 (left) and L4 (right) glands are shown.

**[0065] FIG. 15A.** Neutrophil infiltration goes back to normal levels after clearance of the double mutant  $\Delta 720\Delta hemB$ . Mice were infected as described in materials and methods, and a control group (PBS) of mice received a sterile PBS injection. Glands were harvested at the indicated times, homogenized and kinetically assayed for MPO activity as described in materials and methods. Columns represent means of MPO Units of a group of 6 glands (4 for the PBS control) adjusted by gram of gland, and error bars illustrate standard deviation. Statistical significance between the Day 4 and 12 groups post infection is shown by (Φ) symbol. One-Way ANOVA and Tukey's multiple comparison tests were used (ΦΦ:  $P \leq 0.01$ ; NS: No significant difference between

groups).

**[0066] FIGs. 15B-C.** Immunization of mice with the live-attenuated double mutant ( $\Delta 720\Delta hemB$ ) stimulates a strong humoral response against *S. aureus* bovine mastitis isolates of commonly found *spa* types. Mice were immunized as previously described: serums were collected before priming immunization (Preimmune) and ten days after the boost immunization (Immune). FIG. 15B. IgG titers rise with increasing doses of the live-attenuated strain  $\Delta 720G\Delta hemB$ : each dot represents the total IgG titer of one mouse against a  $\Delta 720G\Delta hemB$  whole cell extract. Medians are represented by thick lines for Immune titers and dashed lines for Preimmune titers. Titers were compared to their corresponding preimmune titers (Two-way ANOVA and Tukey's multiple comparisons test: \*\*\*\*;  $P \leq 0.0001$ ). FIG. 15C. Immunization with the live-attenuated mutant  $\Delta 720G\Delta hemB$  confers IgG titers against components that are shared by mastitis strains of commonly found *spa* types. Each dot represents the total IgG titer of one mouse against the whole cell extract of the indicated strain. Medians are represented by thick lines for Immune titers and dashed lines for Preimmune titers. All immune titers were compared to their corresponding preimmune titer ( $P \leq 0.0001$ ) and between clinical strains (Two-way ANOVA and Sidak's multiple comparisons test: NS: no significant difference).

**[0067] FIG. 16.** shows total serum IgG titers against SACOL0029-1867 fusion protein of mice immunised with the protein mix (composed of 5  $\mu$ g of the antigens SACOL0029, SACOL0442, SACOL0720, and the SACOL0029-1867 fusion),  $10^5$  CFU of the attenuated live strain  $\Delta 720\Delta hemB$  alone or a combination of the protein mix and the  $\Delta 720\Delta hemB$  strain. Open circles ( $\circ$ ) represent data for preimmune titers, black squares ( $\blacksquare$ ) represent data for the immune titers. Each symbol represents the titer for one mouse. Horizontal lines represent the medians: black lines represent the medians for the immune serums while dashed lines represent the medians for the preimmune serums. Titers for the vaccinated mice in the protein mix group and combination group are higher than the titers for the preimmune mice ( $P < 0.001$ ). Statistical significance between immune titers of combination versus the two other vaccinated mice groups is shown (\*\*:  $P < 0.01$ ).

**[0068] FIG. 17.** shows total serum IgG titers against SACOL0029 of mice immunised with the protein mix (composed of 5  $\mu$ g of the antigens SACOL0029, SACOL0442, SACOL0720, and the SACOL0029-1867 fusion),  $10^5$  CFU of the attenuated live strain  $\Delta 720\Delta hemB$  alone or a combination of the protein mix and the  $\Delta 720\Delta hemB$  strain. Open circles ( $\circ$ ) represent data for preimmune titers, black squares ( $\blacksquare$ ) represent data for the immune titers. Each symbol represents the total IgG titer for one mouse. Horizontal lines represent the medians: black lines represent the medians for the immune serums while dashed lines represent the medians for the preimmune serums. Statistical significance between immune and preimmune titers of the three mice groups is shown (\*\*:  $P < 0.01$ ).

**[0069] FIG. 18.** shows total serum IgG titers against the staphylococcal surface protein ClfA for mice immunised with the SACOL0029, SACOL0442, SACOL0720, and SACOL0029-1867 protein mix,  $10^5$  CFU of the attenuated live strain  $\Delta 720\Delta hemB$  alone or a combination of the protein mix and  $\Delta 720\Delta hemB$ . Open circles ( $\circ$ ) represent data for preimmune titers, black squares ( $\blacksquare$ ) represent data for the immune titers. Each symbol represents the titer for one mouse. Horizontal lines represent the medians: black lines represent the medians for the immune serums while dashed lines represent the medians for the preimmune serums. Statistical significance

between preimmune titers and immune titers is shown (\*:  $P < 0.05$ ).

**[0070] FIG. 19.** below shows serum ratio of IgG2a/IgG1 titers against the SACOL0029-1867 fusion polypeptide for mice immunised with the protein mix, or the combination of the protein mix and the attenuated  $\Delta 720\Delta hemB$  live strain.

**[0071] FIG. 20.** shows serum ratios against the SACOL0029 antigen. Open squares ( $\square$ ) represent data for preimmune titers, black squares ( $\blacksquare$ ) represent data for the immune titers. Each symbol represents the titer ratio for one mouse. Horizontal lines represent the medians. Statistical significance between the protein mix group versus combination group ratios is shown (\*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ).

**[0072] FIGs. 21A-J.** I. SACOL0029 polynucleotides (full length sequence SEQ ID NO: 4) and polypeptides (full length, fragment(s) and variant(s) sequences SEQ ID NOs: 5 to 9). Selected epitopes are shown shaded and/or bolded; II. SACOL0720 polynucleotides (full length sequence SEQ ID NO: 10) and polypeptides (full length, fragments and variant(s) sequences SEQ ID NOs: 11 to 27). Selected epitopes are shown shaded; III. SACOL0442 polynucleotides (full length sequence SEQ ID NO: 28) and polypeptides (full length, fragments and variant(s) sequences SEQ ID NOs: 29 to 36 and 1). Selected epitopes are shown shaded; IV. SACOL1867 polynucleotides (full length sequence SEQ ID NO: 37) and polypeptides (full length, fragment(s) and variant(s) sequences SEQ ID NOs: 38 to 41). Selected epitopes are shown shaded. Predicted transmembrane (<http://www.enzim.hu/hmmtop/html/submit.html>) domain shown bolded; V. SACOL1912 polynucleotide (full length sequence SEQ ID NO: 42) and polypeptides (full length and variant(s) sequences SEQ ID NOs: 43 to 44). Selected epitopes are shown shaded (see e.g., SEQ ID NOs: 45-48); VI. SACOL2385 polynucleotide (full length SEQ ID NO: 49) and polypeptides (full length and variant(s) sequences SEQ ID NOs: 50 to 51). Selected epitopes are shown shaded (see e.g., SEQ ID NOs: 52-53); VII. Fusions: (i) SACOL0029-1867 fusion polynucleotide sequences (SEQ ID NOs: 54 and 56) and polypeptide sequences (SEQ ID NOs: 55, 57-58). In the polynucleotide and polypeptide sequences, the double underlined sequence, if any, is that of the polyhistidine, the italicized sequence is the sequence of the SACOL0029 fragment, the single underlined sequence is the sequence of the linker and the bolded sequence is the sequence of the SACOL1867 fragment; (ii) SACOL0720-720 fusion polypeptide sequence (SEQ ID NO: 27) In the polypeptide sequence, the double underlined sequence, if any, is that of the polyhistidine, the italicized sequences are the sequences of the SACOL0720 fragments and the single underlined sequence is the sequence of the linker; (iii) SACOL0442-720 fusion polypeptide sequence (SEQ ID NO: 3). In the polynucleotide and polypeptide sequences, the double underlined sequence, if any, is that of the polyhistidine, the italicized sequence is the sequence of the SACOL0442 fragment, the single underlined sequence is the sequence of the linker and the bolded sequence is the sequence of the SACOL0720 fragment; and VIII. Sequences of linkers (SEQ ID NOs: 59 to 70).

**[0073] FIGs. 22A-D** I. Multiple polynucleotide sequences (SEQ ID NOs: 71-72, 28, 73 to 81) alignment for full length SACOL0442 and orthologues; II. Multiple polypeptide sequences (SEQ ID NOs: 29 and 82 to 92) alignment for full length SACOL0442, orthologues and consensus sequences derived therefrom are presented. In these sequences, "\*" denotes that the residues in that column are identical in all sequences of the alignment, ":" denotes that conserved substitutions have been observed, and "." denotes that semi-conserved substitutions

have been observed. Consensus sequences derived from these alignments are also presented wherein X is any amino acid. In the polypeptide sequences, selected epitopes are shown shaded (see e.g., SEQ ID NOs: 1, 34, 93-97).

**[0074] FIGs. 23A-K** I. Multiple polynucleotide sequences (SEQ ID NOs: 98 to 104, 10, and 105 to 108) alignment for full length SACOL0720 and orthologues; II. Multiple polypeptide sequences (SEQ ID NOs: 11 and 109 to 120) alignment for full length SACOL0720, orthologues. and consensus sequences derived therefrom are presented. In these sequences, “\*” denotes that the residues in that column are identical in all sequences of the alignment, “:” denotes that conserved substitutions have been observed, and “.” denotes that semi-conserved substitutions have been observed. Consensus sequences derived from these alignments are also presented wherein X is any amino acid. In the polypeptide sequences, selected epitopes are shown shaded (see e.g., SEQ ID NOs: 22, 19 and 21).

**[0075] FIG. 24** Multiple polypeptide sequences (SEQ ID NOs: 5 and 121 to 131) alignment for full length SACOL0029, orthologues. and consensus sequences derived therefrom are presented. In these sequences, “\*” denotes that the residues in that column are identical in all sequences of the alignment, “:” denotes that conserved substitutions have been observed, and “.” denotes that semi-conserved substitutions have been observed. Consensus sequences derived from these alignments are also presented wherein X is any amino acid. In the polypeptide sequences, selected epitopes are shown shaded (see e.g., SEQ ID NOs: 132-139). Bolded epitope identified by BCPred™. Shaded epitopes identified by AAp predictions.

**[0076] FIGs. 25A-D.** I-Multiple polynucleotide sequences (SEQ ID NOs: 140 to 151) alignment for full length SACOL1867 and orthologues; II- Multiple polypeptide sequences (SEQ ID NOs: 152 to 164) alignment for full length SACOL1867, orthologues. and consensus sequences derived therefrom are presented. In these sequences, “\*” denotes that the residues in that column are identical in all sequences of the alignment, “:” denotes that conserved substitutions have been observed, and “.” denotes that semi-conserved substitutions have been observed. Consensus sequences derived from these alignments are also presented wherein X is any amino acid. In the polypeptide sequences, selected epitopes are shown shaded (see e.g., SEQ ID NOs: 165-180) and the end of the signal peptide domain and/or transmembrane domain is marked with a line (separates signal peptide and/or transmembrane domain from secreted form).

**[0077] FIG. 26.** I - polynucleotide sequence (SEQ ID NO: 181) for full length SACOL1715 (*hemB*); and II-amino acid sequence (SEQ ID NO: 182) for full length SACOL1715 (*hemB*).

**[0078] FIG. 27.** I - polynucleotide sequence (SEQ ID NO: 183) for full length ClfA (NWMN\_0756, newman); and II-amino acid sequence (SEQ ID NO: 184) for full length ClfA.

#### DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

**[0079]** The present invention showed that a fusion of two antigens created an unexpected synergy in the immune response.

**[0080]** In addition, the present invention also stabilized the SCV phenotype of a *Staphylococcus* via *hemB*

(complete deletion thus impairing the possibility of reversion to an invasive phenotype (Tuchscherr, 2011)) enabling its use as a vaccine delivery system. HemB is coding for the HemB protein/monomer, which combines to create the porphobilinogen synthase or aminolevulinate dehydratase enzyme [EC 4.2.1.24]. In addition, further attenuation was brought about by inactivation of an antigen of the present invention, namely gene SACOL0720, which has been previously shown to be important for *S. aureus* in cationic peptide resistance (Falord, 2012; Kawada-Matsuo, 2011; Meehl, 2007) and *in vivo* during IMI (Allard, 2013). Hence, this attenuated double mutant strain expressing constructs of the present invention is usable for immunization and protection against IMIs.

### **General Definitions**

**[0081]** Headings, and other identifiers, e.g., (a), (b), (i), (ii), etc., are presented merely for ease of reading the specification and claims. The use of headings or other identifiers in the specification or claims does not necessarily require the steps or elements be performed in alphabetical or numerical order or the order in which they are presented.

**[0082]** In the present description, a number of terms are extensively utilized. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

**[0083]** The use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one” but it is also consistent with the meaning of “one or more”, “at least one”, and “one or more than one”.

**[0084]** Throughout this application, the term “about” is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value. In general, the terminology “about” is meant to designate a possible variation of up to 10%. Therefore, a variation of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10% of a value is included in the term “about”. Unless indicated otherwise, use of the term “about” before a range applies to both ends of the range.

**[0085]** As used in this specification and claim(s), the words “**comprising**” (and any form of comprising, such as “comprise” and “comprises”), “**having**” (and any form of having, such as “have” and “has”), “**including**” (and any form of including, such as “includes” and “include”) or “**containing**” (and any form of containing, such as “contains” and “contain”) are inclusive or open-ended and do not exclude additional, un-recited elements or method steps.

**[0086]** As used herein, the term “**consists of**” or “**consisting of**” means including only the elements, steps, or ingredients specifically recited in the particular claimed embodiment or claim.

### **Polypeptides, nucleic acids and delivery systems**

**[0087]** As used herein, the term “vaccine” refers to any compound/agent (“vaccine component”), or combinations thereof, capable of inducing/eliciting an immune response in a host and which permits to treat and/or prevent an infection and/or a disease. Therefore, non-limiting examples of such agent include proteins, polypeptides, protein/polypeptide fragments, immunogens, antigens, peptide epitopes, epitopes, mixtures of proteins, peptides or epitopes as well as nucleic acids, genes or portions of genes (encoding a polypeptide or

protein of interest or a fragment thereof) added separately or in a contiguous sequence such as in nucleic acid vaccines, and the like.

**[0088]** In an aspect of the present invention, there is provided a fusion construct of formula I:

**[0089]** X-A-linker-B-Z (formula (I)),

**[0090]** Wherein A and B are identical or different and are each independently an antigenic polypeptide (i.e. native, fragment or variant thereof) of the present invention.

**[0091]** In a specific embodiment, A and/or B is (a) a polypeptide comprising a SACOL0029 polypeptide as set forth in any one of the sequences depicted in FIG. 24 (SEQ ID NOs: 5 and 121 to 131), a SACOL0264 polypeptide (SEQ ID NO : 185), a SACOL0442 polypeptide as set forth in any one of the sequences depicted in FIG. 22D (SEQ ID NOs: 29 and 82 to 92), a SACOL0718 polypeptide (SEQ ID NO : 186), a SACOL0720 polypeptide as set forth in any one of the sequences depicted in FIGs. 23I-J (SEQ ID NOs: 11 and 109 to 120), a SACOL1353 polypeptide (SEQ ID NO : 187), a SACOL1416 polypeptide (SEQ ID NO : 188), SACOL1611 (SEQ ID NO : 189), a SACOL1867 polypeptide as set forth in any one of the sequences depicted in FIG. 25D (SEQ ID NOs: 152 to 164), a SACOL1912 polypeptide as set forth in FIG. 21G-V (SEQ ID NO : 43), SACOL1944 (SEQ ID NO : 190), a SACOL2144 polypeptide (SEQ ID NO : 191), a SACOL2365 polypeptide (SEQ ID NO : 192), a SACOL2385 polypeptide as set forth in VI on FIG. 21H (SEQ ID NO : 50) or a SACOL2599 polypeptide (SEQ ID NO : 193). In a specific embodiment, the above polypeptide (a) is the secreted or extracellular fragment of the polypeptide defined above. Transmembrane domains can be predicted using, for example, the software TMpred™ (ExPASy) [http://www.ch.embnet.org/software/TMPRED\\_form.html](http://www.ch.embnet.org/software/TMPRED_form.html), <http://www.psort.org/psortb/index.html> <http://www.enzim.hu/hmmtop/html/submit.html> and/or *SignalP* 4.1 (<http://www.cbs.dtu.dk/services/SignalP>). TMpred™ and *SignalP* 4.1 predicted extracellular domain for: SACOL0720: AA 310-508; SACOL0442 AA 36 to 203. Enzim predicted a transmembrane domain SACOL1867 (1-40) so that extracellular domain was: AA 41-239 while <http://www.psort.org/psortb/index.html> predicted that SACOL1867 was an extracellular protein. Since the above-mentioned transmembrane and/or signal peptide domains are putative, the present invention encompasses cases where the antigens presented herein (e.g., SACOL1867) have or not a signal peptide and/or transmembrane domain and encompasses the corresponding extracellular fragments. In an embodiment, the above-mentioned polypeptide is a polypeptide normally secreted or expressed at the surface of the bacteria (e.g., *Staphylococcus aureus*).

**[0092]** The Genbank™ accession numbers for *S. aureus* genes listed herein and their encoded antigenic polypeptides encompassed by the present invention are depicted in Table I below:

**Table I:** Genbank™ accession numbers for the IMI-associated *S. aureus* genes and encoded polypeptides described herein

| Gene name | GenBank™ Gene ID No. | GenBank™ protein No. |
|-----------|----------------------|----------------------|
| SACOL0029 | 3236748              | YP_184940.1          |

|           |         |                                                 |
|-----------|---------|-------------------------------------------------|
|           |         | (SEQ ID NO: 5)                                  |
| SACOL0100 | 3236858 | YP_185004.1                                     |
| SACOL0101 | 3236840 | YP_185005.1                                     |
| SACOL0105 | 3236844 | YP_185009.1                                     |
| SACOL0148 | 3236734 | YP_185048.1                                     |
| SACOL0154 | 3238707 | YP_185054.1                                     |
| SACOL0204 | 3236774 | YP_185103.1                                     |
| SACOL0205 | 3236775 | YP_185104.1                                     |
| SACOL0264 | 3236683 | YP_185159.1<br>WP_000570071<br>(SEQ ID NO: 185) |
| SACOL0442 | 3236485 | YP_185332.1<br>(SEQ ID NO: 29)                  |
| SACOL0461 | 3236475 | YP_185351.1                                     |
| SACOL0608 | 3236353 | YP_185493.1                                     |
| SACOL0660 | 3238251 | YP_185544.1                                     |
| SACOL0688 | 3236721 | YP_185570.1                                     |
| SACOL0690 | 3236723 | YP_185572.1                                     |
| SACOL0704 | 3236241 | YP_185586.1                                     |
| SACOL0718 | 3236599 | YP_185600.1<br>WP_000985996<br>(SEQ ID NO: 186) |
| SACOL0720 | 3236600 | YP_185601.1<br>(SEQ ID NO: 11)                  |
| SACOL0829 | 3238649 | YP_185703.1                                     |
| SACOL1054 | 3236163 | YP_185919.1                                     |
| SACOL1142 | 3236098 | YP_186005.1                                     |
| SACOL1145 | 3237661 | YP_186008.1                                     |

|           |         |                                                 |
|-----------|---------|-------------------------------------------------|
| SACOL1320 | 3236394 | YP_186175.1                                     |
| SACOL1353 | 3236077 | YP_186206.1<br>WP_000603968<br>(SEQ ID NO: 187) |
| SACOL1416 | 3236563 | YP_186268.1<br>WP_000548932<br>(SEQ ID NO: 188) |
| SACOL1611 | 3236575 | YP_186451.1<br>WP_001095260<br>(SEQ ID NO: 189) |
| SACOL1637 | 3238018 | YP_186477.1                                     |
| SACOL1680 | 3238476 | YP_186520.1                                     |
| SACOL1781 | 3236594 | YP_186614.1                                     |
| SACOL1812 | 3238705 | YP_186645.1                                     |
| SACOL1867 | 3236101 | YP_186695.1<br>(SEQ ID NO: 38)                  |
| SACOL1912 | 3236086 | YP_186737.1<br>(SEQ ID NO: 43)                  |
| SACOL1944 | 3237515 | YP_186769.1<br>WP_000149064<br>(SEQ ID NO: 190) |
| SACOL2092 | 3238693 | YP_186907.1                                     |
| SACOL2144 | 3237436 | YP_186957.1<br>WP_000908177<br>(SEQ ID NO: 191) |
| SACOL2169 | 3237416 | YP_186981.1                                     |
| SACOL2171 | 3237418 | YP_186983.1                                     |
| SACOL2321 | 3238070 | YP_187128.1                                     |

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|           |         |                                                 |
|-----------|---------|-------------------------------------------------|
| SACOL2325 | 3238483 | YP_187132.1                                     |
| SACOL2342 | 3235997 | YP_187148.1                                     |
| SACOL2365 | 3238203 | YP_187170.1<br>WP_000827000<br>(SEQ ID NO: 192) |
| SACOL2379 | 3237628 | YP_187183.1                                     |
| SACOL2385 | 3238646 | YP_187189.1<br>(SEQ ID NO: 50)                  |
| SACOL2599 | 3237186 | YP_187390.1<br>AAW38600<br>(SEQ ID NO: 193)     |

**[0093]** Consensuses derived from the alignments of certain the above listed polypeptides are presented in FIGs. 21-25. In specific embodiment of these consensuses, each X in the consensus sequences (e.g., consensuses in FIGs. 21-25) is defined as being any amino acid, or absent when this position is absent in one or more of the orthologues presented in the alignment. In specific embodiment of these consensuses, each X in the consensus sequences is defined as being any amino acid that constitutes a conserved or semi-conserved substitution of any of the amino acid in the corresponding position in the orthologues presented in the alignment, or absent when this position is absent in one or more of the orthologues presented in the alignment. In FIGs. 21-25, conservative substitutions are denoted by the symbol “.” and semi-conservative substitutions are denoted by the symbol “.”. In another embodiment, each X refers to any amino acid belonging to the same class as any of the amino acid residues in the corresponding position in the orthologues presented in the alignment, or absent when this position is absent in one or more of the orthologues presented in the alignment. In another embodiment, each X refers to any amino acid in the corresponding position of the orthologues presented in the alignment, or absent when this position is absent in one or more of the orthologues presented in the alignment. In a specific embodiment, A and/or B is a polypeptide satisfying any one of these consensuses or a fragment thereof.

**[0094]** Conservative amino acid mutation may include addition, deletion, or substitution of an amino acid; a conservative amino acid substitution is defined herein as the substitution of an amino acid residue for another amino acid residue with similar chemical properties (e.g., size, charge, or polarity). Such a conservative amino acid substitution may be a basic, neutral, hydrophobic, or acidic amino acid for another of the same group (see e.g., Table II below). By the term "basic amino acid" it is meant hydrophilic amino acids having a side chain pK value of greater than 7, which are typically positively charged at physiological pH. Basic amino

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acids include histidine (His or H), arginine (Arg or R), and lysine (Lys or K). By the term "neutral amino acid" (also "polar amino acid"), it is meant hydrophilic amino acids having a side chain that is uncharged at physiological pH, but which has at least one bond in which the pair of electrons shared in common by two atoms is held more closely by one of the atoms. Polar amino acids include serine (Ser or S), threonine (Thr or T), cysteine (Cys or C), tyrosine (Tyr or Y), asparagine (Asn or N), and glutamine (Gln or Q). The term "hydrophobic amino acid" (also "non-polar amino acid") is meant to include amino acids exhibiting a hydrophobicity of greater than zero according to the normalized consensus hydrophobicity scale of Eisenberg (1984). Hydrophobic amino acids include proline (Pro or P), isoleucine (Ile or I), phenylalanine (Phe or F), valine (Val or V), leucine (Leu or L), tryptophan (Trp or W), methionine (Met or M), alanine (Ala or A), and glycine (Gly or G). "Acidic amino acid" refers to hydrophilic amino acids having a side chain pK value of less than 7, which are typically negatively charged at physiological pH. Acidic amino acids include glutamate (Glu or E), and aspartate (Asp or D).

**[0095]** A semi-conserved amino acid replaces one residue with another one that has similar steric conformation, but does not share chemical properties. Examples of semi-conservative substitutions would include substituting cysteine for alanine or leucine; substituting serine for asparagine; substituting valine for threonine; or substituting proline for alanine.

**[0096]** The Table II below indicates which amino acid belongs to each amino acid class.

| Class                                  | Name of the amino acids                                 |
|----------------------------------------|---------------------------------------------------------|
| Aliphatic                              | Glycine, Alanine, Valine, Leucine, Isoleucine           |
| Hydroxyl or Sulfur/Selenium-containing | Serine, Cysteine, Selenocysteine, Threonine, Methionine |
| Cyclic                                 | Proline                                                 |
| Aromatic                               | Phenylalanine, Tyrosine, Tryptophan                     |
| Basic                                  | Histidine, Lysine, Arginine                             |
| Acidic and their Amide                 | Aspartate, Glutamate, Asparagine, Glutamine             |

**[0097]** The similarity and identity between amino acid or nucleotide sequences can be determined by comparing each position in the aligned sequences. Optimal alignment of sequences for comparisons of similarity and/or identity may be conducted using a variety of algorithms, for example using a multiple sequence alignment program/software well known in the art such as ClustalW™, SAGA™, UGENE™ or T-coffee™. Examples of multiple sequence alignments are described in the examples below and depicted in FIGs. 21A to 25.

#### Gene operon

**[0098]** In another embodiment, A and/or B is (b) a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a) as defined above. For example, SACOL0718 is a gene from the same operon as SACOL0720.

#### Fragment

**[0099]** In another embodiment, A and/or B is (c) a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a) or (b) as defined above.

**[00100]** An immunogenic fragment of a protein/polypeptide is defined as a part of a protein/polypeptide which is capable of inducing/eliciting an immune response in a host. In an embodiment, the immunogenic fragment is capable of eliciting the same immune response in kind, albeit not necessarily in amount, as the protein/polypeptide. An immunogenic fragment of a protein/polypeptide preferably comprises one or more epitopes of said protein/polypeptide. An epitope of a protein/polypeptide is defined as a fragment of said protein/polypeptide of at least about 4 or 5 amino acids in length, capable of eliciting a specific antibody and/or an immune cell (e.g., a T cell or B cell) bearing a receptor capable of specifically binding said epitope. Two different kinds of epitopes exist: linear epitopes and conformational epitopes. A linear epitope comprises a stretch of consecutive amino acids. A conformational epitope is typically formed by several stretches of consecutive amino acids that are folded in position and together form an epitope in a properly folded protein. An immunogenic fragment as used herein refers to either one, or both, of said types of epitopes. In an embodiment where immunogenic fragments are used alone (i.e. not fused in a larger polypeptide construct (e.g., fusion with other antigenic fragment)), the immunogenic fragment of a protein/polypeptide comprises at least 16 amino acid residues. In a further embodiment, the immunogenic fragment comprises at least 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, or 160 consecutive amino acids of the native protein/polypeptide. In a specific embodiment, the fragment has at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or 50 or more consecutive amino acids of the native protein/polypeptide. In an embodiment where the at least one immunogenic fragment forms part of a larger polypeptide construct (e.g., fusion with other antigenic polypeptide, fragment or variant thereof), the immunogenic fragment comprises at least 13 consecutive amino acid residues of the polypeptide. Without being so limited, fragments encompassed by the present invention comprise immunogenic fragments of at least 13 consecutive amino acids of SACOL029 as shown in FIG. 21 (and corresponding fragments in SACOL029 orthologues (e.g., depicted in FIG. 24); of SACOL0442 as shown in FIG. 21 (and corresponding fragments in SACOL0442 orthologues (e.g., depicted in FIG. 22); of SACOL0720 as shown in FIG. 21, (and corresponding fragments in SACOL0720 orthologues (e.g., depicted in FIG. 23); and of SACOL1867 as shown in FIG. 21 (and corresponding fragments in SACOL1867 orthologues (e.g., depicted in FIG. 25). In another embodiment, fragments encompassed by the present invention include immunogenic fragments comprising at least one epitope of antigenic proteins/polypeptides of the present invention (polypeptide (a) defined above). In another embodiment, fragments encompassed by the present invention include immunogenic fragments comprising at least one epitope as depicted (shaded) in any one of the antigenic proteins/polypeptides depicted in any one of FIGs. 21 to 25. Without being so limited, epitopes in a sequence may be predicted with softwares such as BCPred™, AAP™, FBCPred™ and ABCPred™.

**[00101]** In an embodiment, the above-mentioned immunogenic fragment comprises a sequence that is conserved (i.e. identical) in at least two different strains of *Staphylococcus aureus*. In further embodiments, the

above-mentioned immunogenic fragment comprises a sequence that is conserved (i.e. identical) in at least 3, 4, 5, 6, 7, 8, 9 or 10 different strains of *Staphylococcus aureus*. In another embodiment, the above-mentioned strains of *Staphylococcus aureus* are COL, RF122, NCTC 8325, JH1, JH9, Newman, Mu3, Mu50, USA300-FPR3757, N315, MW2 or MSSA476. In an embodiment, the above-mentioned strains of *Staphylococcus aureus* are associated with bovine mastitis (e.g., RF122).

#### Variants

**[00102]** In another embodiment, the above-mentioned polypeptide, or a polypeptide substantially identical to said polypeptide, is expressed in at least two different strains of *Staphylococcus aureus*. Substantially identical as used herein refers to polypeptides having at least 60% of identity, in embodiments at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% of identity in their amino acid sequences. In further embodiments, the polypeptides have at least 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% of identity in their amino acid sequences with other polypeptides to which they are compared.

**[00103]** In another embodiment, A and/or B is **(d)** a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a) to (c) defined above. In other embodiments, the amino acid is at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to (a) (e.g., over their full length). In further embodiments, the amino acid is at least 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% to (a). For example, antigens orthologues presented in alignments of FIGs. 21-25 are not identical but present a certain identity with the antigens or fragments to which they are compared. Consensuses presented in these FIGs embody such percent identities.

**[00104]** In another embodiment, A and/or B is **(e)** a polypeptide comprising an immunogenic variant comprising at least 13 consecutive amino acids of any one of (a) to (d). An immunogenic variant of a protein/polypeptide is defined as a part of a protein/polypeptide which is capable of inducing/eliciting an immune response in a host. As will be understood by the person of ordinary skill, agents (proteins/polypeptides, fragments thereof) having non-naturally occurring modifications (e.g., immunogenic variants) and which are capable of inducing an immune response specific for the unmodified agent (e.g., capable of inducing the production of antibodies capable of recognizing the unmodified agent) are also within the scope of the term "vaccine component". For example, the vaccine components of the present invention can be modified to enhance their activity, stability, and/or bioavailability, and/or to reduce their toxicity. Conservative amino acid substitutions may be made, like for example replacement of an amino acid comprising an acidic side chain by another amino acid comprising an acidic side chain, replacement of a bulky amino acid by another bulky amino acid, replacement of an amino acid comprising a basic side chain by another amino acid comprising a basic side chain, and the like. A person skilled in the art is well able to generate variants of a protein/polypeptide. This is for instance done through screening of a peptide library or by peptide changing programs. An immunogenic variant according to the invention has essentially the same immunogenic properties of said protein in kind, not necessarily in amount. An immunogenic variant of a protein/polypeptide of the invention may for instance comprise a fusion protein and/or chimeric

protein. For example, the biological function of a protein identified herein predicted to be an exotoxin, enterotoxin or superantigen (e.g., SACOL0442) could potentially interfere with the mammalian immune system and antibody production, and/or show some toxicity in the host. Although such interference was not observed when the SACOL0442 polypeptide was used in combination with for example SACOL0720 during immunization, it may be useful to modify the protein or polypeptide used for vaccination so that the biological activity of the exotoxin is decreased. For such a purpose, it is possible to inactivate the exotoxin with chemicals (e.g., formaldehyde). It is also possible to use molecular biology techniques to delete or mutate the putative region(s) involved in exotoxin activity without losing immunogenicity (Chang *et al.*, 2008). Another example is the conjugation or mixture of amino acid-based components with nucleic acids (e.g., genes or portions of genes added separately or in a contiguous sequence) carbohydrates such as those found in microbial polysaccharide capsules or biofilms. Other examples of variants include antigens described herein or fragments thereof comprising at either of their N or C terminus or inserted within their antigen sequence, an oligopeptide useful for purification (e.g., affinity purification) or useful as a spacer or linker. Examples of oligopeptides useful for affinity purification include polyhistidine tags (e.g., 6-10 histidine residues including or not RGS tags (e.g. HHHHHH, RGSHHHHHH, or RGSHHHHHGS). The his-tag may also be followed by an amino acid sequence suitable to facilitate a removal of the polyhistidine-tag using endopeptidases. The "X" and/or "Z" segments as recited in formula (I) also may comprise such oligopeptide useful for purification and/or sequence suitable to facilitate removal of such oligopeptide useful for purification.

**[00105]** In specific embodiments, the immunogenic fragment comprises at least one epitope of the polypeptide (a). Without being so limited, in certain embodiments, the immunogenic fragment comprises at least one epitope of the polypeptide (a) as depicted (shaded) in the sequences presented in FIGs. 21-25. In other specific embodiments, the variants are as disclosed in FIGs. 21-25.

#### Linker

**[00106]** Insertion of linkers between fusion protein domains can increase bioactivity by augmenting distance between domains alleviating potential repulsive forces between different segments (e.g., antigenic fragments) of the construct resulting in improved and/or restored protein folding. Different sequences of polypeptide linkers can be used and are known to have distinct properties, such as flexible, rigid or cleavable linkers. The present invention encompasses the use of any such linkers including any one of those listed in Chen et. al, Adv Drug Deliv Rev. (2013), 65(10):1357-69 for example. Examples herein provide illustrations of specific linkers that were used (i.e. GGGGSGGGGSGGGGS (SEQ ID NO: 60), ERKYK (SEQ ID NO: 61), or and EAAAKEAAK (SEQ ID NO: 62)), i.e. flexible linker structures, rich in small hydrophilic amino acids that maintain distance between the two connected domains and improve their folding.

**[00107]** In another specific embodiment, the Fc comprises a CH2 domain, a CH3 domain and a hinge region. In another specific embodiment, the Fc is a constant domain of an immunoglobulin selected from the group consisting of IgG-1, IgG-2, IgG-3, IgG-3 and IgG-4. In another specific embodiment, the Fc is a constant domain of an immunoglobulin IgG-1.

**[00108]** Linkers may be included between contiguous antigens of the fusion (e.g., 1 linker in fusion comprising

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two antigens, 2 linkers in fusions comprising three antigens, three linkers in fusions comprising four antigens, etc.). In fusions where large protein domains are used, linker may be larger and may comprise a fragment crystallizable region (Fc).

**[00109]** In a specific embodiment, the linker is an amino acid sequence of at least one amino acid or is absent. In a specific embodiment, the linker comprises at least three (at least 4, 5, 6, 7, 8, 9 or 10) amino acids selected from the group consisting of glycine, serine, alanine, aspartate, glutamate and lysine. In a specific embodiment, the linker is (EAAAK)<sub>n</sub> (SEQ ID NO: 63); (GGGGS)<sub>n</sub> (SEQ ID NO: 67); or (XPXPXP)<sub>n</sub> (SEQ ID NO: 69) wherein x is any amino acid; wherein n is any one of 1 to 5, more specifically 1, 2, 3, 4 or 5; EAAAKEAAAK (SEQ ID NO: 62); EAAAKEAAKEAAAK (SEQ ID NO: 64); GGGGS (SEQ ID NO: 67); GGGSGGGGS (SEQ ID NO: 68); GGGSGGGSGGGGS (SEQ ID NO: 60); XPXPXP (SEQ ID NO: 69), wherein x is any amino acid; XPXPXPXPXPXP (SEQ ID NO: 70), wherein x is any amino acid; ERKYK (SEQ ID NO: 61); ERKYKERKYK (SEQ ID NO: 65); ERKYKERKYKERKYK (SEQ ID NO: 66). In a more specific embodiment, the linker is GGGSGGGSGGGGS (SEQ ID NO: 60), ERKYK (SEQ ID NO: 61), or EAAAKEAAAK (SEQ ID NO: 62).

#### N and C terminal of construct

**[00110]** X and Z are each independently absent or an amino acid sequence of at least one amino acid. Without being so limited, they may be one or more of amino acids resulting from cloning strategy, amino acids used to facilitate purification of the construct (e.g. polyhistidine), amino acids suitable to facilitate a removal of the purification-tag using endopeptidases. In specific embodiments, where the fusion construct comprises three or more antigen polypeptides, any one of X and/or Z may also include the sequence of a further antigen (antigen C, antigen D, etc.) and, optionally that of at least one further linker. Such embodiments wherein X and/or Z comprise one or more further antigen(s) and optionally linker(s), could be more specifically illustrated as e.g., formula (II) or (III) as follows X'-C-linker<sub>1</sub>-A-linker<sub>2</sub>-B-Z' (II) when the fusion comprises at least 3 antigens; or X'-C-linker<sub>1</sub>-A-linker<sub>2</sub>-B-linker<sub>3</sub>-D-Z' (III) when the fusion comprises at least 4 antigens. In both formula (II) and (III) X', Z', linker<sub>1</sub>, linker<sub>2</sub>, and, the case being, linker<sub>3</sub>, are identical or different and are independently defined as are X, Z and linker in formula (I) defined herein.

**[00111]** Hence, in specific embodiments, the fusion construct comprises 2, 3, 4 or more antigen polypeptides (and, the case being further linkers). In a more specific embodiment, and without being so limited the fusion construct may be SACOL0029\_SACOL0442; SACOL0029\_SACOL0720; SACOL0029\_SACOL1867; SACOL0029\_SACOL0720\_SACOL1867; SACOL0029\_SACOL1867\_SACOL0442; SACOL0029\_SACOL0720\_SACOL0442; SACOL0442\_SACOL0029\_SACOL0720; SACOL0442\_SACOL0029\_SACOL1867; SACOL0442\_SACOL1867\_SACOL0720; SACOL0720\_SACOL0442\_SACOL1867; or SACOL0029\_SACOL1867\_SACOL0720\_SACOL0442, or any of the foregoing constructs wherein the antigen polypeptides are in any other order.

#### Combination

**[00112]** The constructs of the present invention may be used as sole immunogenic component of a composition (e.g., vaccine) of the present invention or in combination with one or more further fusion construct(s),

immunogenic polypeptide(s), fragment(s) or variant(s) thereof and/or live attenuated bacteria (e.g., *S. aureus*) (expressing or not fusion constructs and/or polypeptide(s), fragment(s) or variant(s) thereof).

**[00113]** The one or more fusion constructs may be any immunogenic fusion construct including a further fusion construct as defined above (see e.g., Example 14).

**[00114]** The one or more immunogenic polypeptide(s), fragment(s) or variant(s) thereof for use in compositions of the present invention may be any polypeptide(s), fragment(s) or variant(s) that contribute to the immunogenicity of the compositions of the present invention as defined herein. Without being so limited, such polypeptide(s), fragment(s) or variant(s) includes (a) a polypeptide comprising a SACOL0029 polypeptide as set forth in any one of the sequences depicted in FIG. 24 (SEQ ID NOs: 5 and 121 to 131), a SACOL0264 polypeptide (SEQ ID NO : 185), a SACOL0442 polypeptide as set forth in any one of the sequences depicted in FIG. 22D (SEQ ID NOs: 29 and 82 to 92), a SACOL0718 polypeptide (SEQ ID NO : 186), a SACOL0720 polypeptide as set forth in any one of the sequences depicted in FIGs. 23I-K (SEQ ID NOs: 11 and 109 to 120), a SACOL1353 polypeptide (SEQ ID NO : 187), a SACOL1416 polypeptide (SEQ ID NO : 188), SACOL1611 (SEQ ID NO : 189), a SACOL1867 polypeptide as set forth in any one of the sequences depicted in FIG. 25D (SEQ ID NOs: 152 to 164), a SACOL1912 polypeptide (SEQ ID NO : 43), a SACOL1944 polypeptide (SEQ ID NO : 190), a SACOL2144 polypeptide (SEQ ID NO : 191), a SACOL2365 polypeptide (SEQ ID NO : 192), a SACOL2385 polypeptide (SEQ ID NO : 50) or a SACOL2599 polypeptide (SEQ ID NO : 193); (b) a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a); (c) a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a) or (b); (d) a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a) to (c); or (e) a polypeptide comprising an immunogenic variant comprising at least 13 consecutive amino acids of any one of (a) to (c), as defined above. Without being so limited, any such polypeptide(s), fragment(s) or variant(s) encompasses those included in compositions (e.g., vaccines #1 to # 8) exemplified in Examples 1 to 14 and 21-26.

#### Live attenuated bacteria

**[00115]** The live attenuated bacteria (e.g. *S. aureus*) for use in compositions of the present invention may be independent from the fusions constructs and/or polypeptide(s), fragment(s) or variant(s) thereof of the present invention, or be the vessel for (i.e. may express) such fusion constructs and/or polypeptide(s), fragment(s) or variant(s) thereof of the present invention.

**[00116]** Without being so limited, as illustrated herein, useful live attenuated bacteria in the context of combinations of the present invention include *Staphylococcus* (e.g., *aureus*) bacteria having at least one gene contributing to virulence (e.g.,  $\Delta 720$ ) or contributing to fitness in the host (e.g., a metabolic gene) mutated or deleted. Without being so limited, such gene may be any one of the genes identified in Novick 2003, Novick 2008, or Maresso and Schneewind 2008.

**[00117]** In a further embodiment, the live attenuated bacteria may be further attenuated by having a stabilized SCV phenotype. As used herein the terms "SCV phenotype" refers to bacteria having a dysfunctional oxidative

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metabolism causing a slow growth, an alteration in the expression of virulence factors, and an ability to be internalized in host cells. As used herein the term « stabilized SCV phenotype» is used to denote an SCV strain retaining the SCV phenotype i.e. unable to produce invasive revertants (*i.e.*, a reversion to the normal growth phenotype). Such stabilized SCV *S. aureus* may be produced by mutating or deleting any one of the genes (*e.g.*,  $\Delta hemB$ ) listed in Table III below. Without being limited, the present invention encompasses the use of the stabilized SCV *S. aureus* exemplified in Examples 15 to 25. Mutation as used herein includes a substitution, a deletion and/or an insertion of one or more nucleotides that prevents expression of the polypeptide encoded by a gene of the present invention or that prevents expression of a functional polypeptide. In a preferred embodiment, the mutation prevents expression of the polypeptide. In another specific embodiment, the two mutations in the same attenuated live or inactivated strain of *S. aureus* are a deletion or an insertion. It is expected that a mutated strain of *S. aureus* having a mutation at any position of one of the genes of the present invention that prevents expression of the polypeptide can be used as an attenuated live vaccine in accordance with the present invention. Attenuated live vaccines, *i.e.* vaccines comprising the bacterium according to the invention in a live attenuated form, have the advantage over inactivated vaccines that they best mimic the natural way of infection. In addition, their replicating abilities allow vaccination with low amounts of bacteria; their number will automatically increase until it reaches the trigger level of the immune system. From that moment on, the immune system will be triggered and will finally eliminate the bacteria. A minor disadvantage of the use of live attenuated bacteria however might be that inherently there is a certain level of virulence left. This need not be a real disadvantage as long as the level of virulence is acceptable, *i.e.* as long as the vaccine at least decreases the bacterial infection (*e.g.*, IMI) symptoms. Of course, the lower the remaining virulence of the live attenuated vaccine is, the less influence the vaccination has on weight gain during/after vaccination.

Table III: Genbank™ accession numbers for *S. aureus* genes associated with SCV phenotype (Kahl, 2014)

| Gene Name | GenBank™ Gene ID No. | GenBank™ Protein No.                                        |
|-----------|----------------------|-------------------------------------------------------------|
| hemB      | 3238571 (SACOL1715)  | AAW36820.1<br>WP_000667126.1<br>GI:446589780<br>EC:4.2.1.24 |
| menB      | 3236546 (SACOL1052)  | AAW36517.1<br>WP_000526687.1<br>GI:446448832<br>EC:2.2.1.9  |
| thyA      | 3238178 (SACOL1462)  | AAW36663.1<br>WP_000667126.1                                |

|                   |                     |                                          |
|-------------------|---------------------|------------------------------------------|
|                   |                     | GI:446589780<br>EC:2.1.1.45              |
| fusA              | 3236183 (SACOL0593) | AAW37703.1<br>GI:57285609                |
| FusE (gene: rplF) | 3238328 (SACOL2224) | AAW37099.1<br>GI:57285005                |
| relA (relA2)      | 3238211 (SACOL1689) | AAW36795.1<br>GI:57284701<br>EC:2.7.6.5  |
| cspB              | 3238398 (SACOI2731) | AAW37379.1<br>GI:57285285                |
| hemH              | 3236274 (SACOI1888) | AAW36901.1<br>GI:57284807<br>EC:4.99.1.1 |
| ctaA              | 3237823 (SACOL1124) | AAW38004.1<br>GI:57285910                |

#### Nucleic acids

**[00118]** The nucleic acid of the present invention preferably comprises a nucleotide sequence that encodes one or more proteins/polypeptides noted above (or fragments thereof) operably linked to regulatory elements needed for gene expression, such as a promoter, an initiation codon, a stop codon, enhancers, and a polyadenylation signal. Regulatory elements are preferably selected that are operable in the species to which they are to be administered. In specific embodiments, the nucleic acid is as depicted in FIGs. 21 to 25.

**[00119]** Within the context of the present invention is the *in vivo* administration of a nucleic acid of the invention to a mammal so that one or more proteins/polypeptides (or a fragment thereof) of interest is/are expressed in the mammal (e.g., nucleic acid vaccine, DNA or RNA vaccine).

#### **Delivery systems**

**[00120]** The nucleic acid of the present vaccine can be "naked" DNA or can be operably incorporated in a vector. Nucleic acids may be delivered to cells *in vivo* using methods well known in the art such as direct injection of DNA, receptor-mediated DNA uptake, viral-mediated transfection or non-viral transfection and lipid-based transfection, all of which may involve the use of vectors. Direct injection has been used to introduce naked DNA

into cells *in vivo* (see e.g., Acsadi *et al.* (1991) *Nature* **332**:815-818; Wolff *et al.* (1990) *Science* **247**:1465-1468). A delivery apparatus (e.g., a "gene gun") for injecting DNA into cells *in vivo* may be used. Such an apparatus may be commercially available (e.g., from BioRad). Naked DNA may also be introduced into cells by complexing the DNA to a cation, such as polylysine, which is coupled to a ligand for a cell-surface receptor (see for example Wu, G. and Wu, C. H. (1988) *J. Biol. Chem.* **263**: 14621; Wilson *et al.* (1992) *J. Biol. Chem.* **267**: 963-967; and U.S. Pat. No. 5,166,320). Binding of the DNA-ligand complex to the receptor may facilitate uptake of the DNA by receptor-mediated endocytosis. A DNA-ligand complex linked to adenovirus capsids which disrupt endosomes, thereby releasing material into the cytoplasm, may be used to avoid degradation of the complex by intracellular lysosomes (see for example Curiel *et al.* (1991) *Proc. Natl. Acad. Sci. USA* **88**: 8850; Cristiano *et al.* (1993) *Proc. Natl. Acad. Sci. USA* **90**:2122-2126).

**[00121]** Useful delivery vectors include biodegradable microcapsules, immuno-stimulating complexes (ISCOMs) or liposomes, and genetically engineered attenuated live vectors such as cells, viruses or bacteria.

**[00122]** Liposome vectors are unilamellar or multilamellar vesicles, having a membrane portion formed of lipophilic material and an interior aqueous portion. The aqueous portion is used in the present invention to contain the polynucleotide material to be delivered to the target cell. It is generally preferred that the liposome forming materials have a cationic group, such as a quaternary ammonium group, and one or more lipophilic groups, such as saturated or unsaturated alkyl groups having about 6 to about 30 carbon atoms. One group of suitable materials is described in European Patent Publication No. 0187702, and further discussed in U.S. Pat. No. 6,228,844 to Wolff *et al.*, the pertinent disclosures of which are incorporated by reference. Many other suitable liposome-forming cationic lipid compounds are described in the literature. See, e.g., L. Stamatatos, *et al.*, *Biochemistry* 27:3917-3925 (1988); and H. Eibl, *et al.*, *Biophysical Chemistry* 10:261-271 (1979). Alternatively, a microsphere such as a polylactide-coglycolide biodegradable microsphere can be utilized. A nucleic acid construct is encapsulated or otherwise complexed with the liposome or microsphere for delivery of the nucleic acid to a tissue, as is known in the art.

**[00123]** Preferred viral vectors include Bacteriophages, Herpes virus, Adenovirus, Polio virus, Vaccinia virus, defective retroviruses, adeno-associated virus (AAV) and Avipox. Methods of transforming viral vector with an exogenous DNA construct are also well described in the art. See Sambrook and Russell, above.

**[00124]** As indicated above, the nucleic acid (e.g., DNA or RNA) may be incorporated in a host such as a host cell *in vitro* or *ex vivo* (e.g., an immune cell such as a dendritic cell) or, as indicated above, in an attenuated microbial host (e.g., attenuated *S. aureus*, SCV, etc., see e.g., Examples 25-26 for instance) by transfection or transformation, and the transfected or transformed cell or microorganism, which expresses the polypeptide (e.g. fusion of multiple antigens or fragments thereof and/or single antigens or fragments thereof) of interest, may be administered to the subject. Following administration, the cell will express the protein or polypeptide of interest (or a variant or fragment thereof) in the subject, which will in turn lead to the induction of an immune response directed against the protein, polypeptide or fragment thereof.

**[00125]** The use of attenuated live bacteria to immunize and/or to deliver specific constructs or antigen mixture

of the present invention represents an interesting approach to improve immune responses (Griffiths and Khader, 2014). Live attenuated organisms that mimic natural infection stimulate the immune system in a powerful manner, eliciting broad and robust immune responses that produce both serum and mucosal antibodies, and effector and memory T cells which act synergistically to protect against disease (Detmer and Glenting, 2006; Kollaritsch et al, 2000; Pasetti et al., 2011). Examples of suitable attenuated live bacterial vectors include *S. aureus*, *Salmonella typhimurium*, *Salmonella typhi*, *Shigella*, *Bacillus*, *Lactobacillus*, Bacille Calmette-Guerin (BCG), *Escherichia coli*, *Vibrio cholerae*, *Campylobacter*, or any other suitable bacterial vector, as is known in the art. Methods of transforming live bacterial vectors with an exogenous DNA construct are well described in the art. See, for example, Joseph Sambrook and David W. Russell, Molecular Cloning, A Laboratory Manual, 3rd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2001). The present invention encompasses the use of a composition comprising an attenuated live bacterium (e.g.,  $\Delta$ hemBA720 *S. aureus* expressing the construct of the present invention as sole immunogenic component or in combination with other attenuated live bacteria each expressing another polypeptide, fragment or variant of the present invention (e.g., SACOL0442, SACOL0720 or fragments or variants thereof).

### Compositions

**[00126]** The polypeptides, nucleic acids and delivery systems (e.g., host cells comprising said nucleic acids or vectors) described herein can be formulated into compositions. As used herein, the term “pharmaceutically acceptable” refers to vaccine components (e.g., excipients, carriers, adjuvants) and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a subject. Preferably, as used herein, the term “pharmaceutically acceptable” means approved by regulatory agency of the federal or state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and in humans. The term “excipient” refers to a diluent, carrier, or vehicle with which the vaccine components of the present invention may be administered. Sterile water or aqueous saline solutions and aqueous dextrose and glycerol solutions may be employed as carriers, particularly for injectable solutions.

**[00127]** In an embodiment, the agent of the present invention is administered in combination with an adjuvant or immunostimulant. Suitable adjuvant or immunostimulant that may improve the efficacy of components to raise an immune response include but is not limited to oils (e.g., mineral oils, emulsified oil such as MONTANIDE™ or EMULSIGENT™-D), metallic salts (e.g., alum, aluminum hydroxide or aluminum phosphate), cationic peptides (Bowdish et al., 2005; Hancock, et al., 2000) such as indolicidin, a cationic peptide produced by the cow's immune cells (Falla et al., 1996), natural and artificial microbial components (e.g., bacterial liposaccharides, Freund's adjuvants, muramyl dipeptide (MDP), cyclic-diguanosine-5'-monophosphate (c-di-GMP), pathogen-associated molecular patterns (PAMPS) such as surface polysaccharides, lipopolysaccharides, glycans, peptidoglycan or microbial DNA (e.g., CpG), plant components such as saponins (e.g., Quil-A™), and/or one or more substances that have a carrier effect (e.g., bentonite, latex particles, liposomes, ISCOM™, DNA and polyphosphazine (PCPP) copolymers). Immunization with synthetic nanoparticles (such as those made from a biodegradable synthetic polymer like poly(D,L-lactico-glycolic acid)) containing antigens plus ligands that signal

through TLR to stimulate proinflammatory cytokines is also possible (Kasturi et al, 2011).

**[00128]** Vaccine components of the invention may be administered in a pharmaceutical composition. Pharmaceutical compositions may be administered in unit dosage form. Any appropriate route of administration may be employed, for example, parenteral, subcutaneous, intramuscular, intramammary, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraarticular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, or oral administration. Examples of specific routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, intramammary; oral (e.g., inhalation); transdermal (topical); transmucosal, and rectal administration.

**[00129]** Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer such vaccine components with or without adjuvants to subjects. Methods well known in the art for making pharmaceutical compositions and formulations are found in, for example, Remington: The Science and Practice of Pharmacy, (20<sup>th</sup> ed.) ed. A. R. Gennaro A R., 2000, Lippincott: Philadelphia. Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, miglyol, oils of vegetable origin, or hydrogenated naphthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for compounds of the invention include ethylenevinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation or intramammary injection may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, miglyol, glycocholate and deoxycholate, or may be oily solutions (e.g., paraffin oil) for administration in the form of nasal drops, or as a gel.

**[00130]** Therapeutic formulations may be in the form of liquid solutions or suspension; for oral administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols. Solutions or suspensions used for parenteral, intradermal, intramammary or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils (e.g., paraffin oil), polyethylene glycols, glycerin, propylene glycol, miglyol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; reducing agents such as dithiothreitol, buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

**[00131]** Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous or intramammary administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor™ ELTM (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS).

[00132] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets or feed. For the purpose of oral vaccine administration, the active components can be incorporated with excipients and used in the form of tablets, troches, capsules or in feed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel™, or corn starch; a lubricant such as magnesium stearate or sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[00133] For administration by inhalation, the vaccine components are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

[00134] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[00135] Liposomal suspensions (including liposomes targeted to specific cell types) can also be used as pharmaceutically acceptable carriers.

[00136] The pharmaceutical compositions may also contain preserving agents, solubilizing agents, stabilizing agents, wetting agents, emulsifiers, sweeteners, colorants, odorants, salts for the variation of osmotic pressure, buffers, coating agents or antioxidants. They may also contain other therapeutically valuable agents.

[00137] Intravenous, intramuscular, subcutaneous, intramammary or oral administration is a preferred form of use. The dosages in which the components of the present invention are administered in effective amounts depend on the nature of the specific active ingredient, the host and the requirements of the subject and the mode of application.

#### **Microbial Targets**

[00138] Polypeptides, nucleic acids and delivery systems of the present invention may be used as antimicrobial agents against Staphylococcal infections including those causing intramammary infection (IMI). In a preferred embodiment, the Staphylococcal infections are caused by *Staphylococcus aureus*.

#### **Methods of immunizing with polypeptides, nucleic acids, vectors, cells, compositions and delivery systems**

[00139] Encompassed by the methods, uses, pharmaceutical compositions and kits of the present invention is passive and active immunization.

**[00140]** Passive immunization is the injection of antibodies or antiserum, previously generated against the pathogen (or antigens described herein), in order to protect or cure a recipient animal of an infection or future infection. Protection fades over the course of a few weeks during which time the active immunization with polypeptides, nucleic acids or delivery systems (e.g., as described above) will have time to generate a lasting protective response. Serum for passive immunization can be generated by immunization of donor animals using the polypeptides, nucleic acids or delivery systems, as described herein. This serum, which contains antibodies against the antigens, can be used immediately or stored under appropriate conditions. It can be used to combat acute infections (e.g., IMI) or as a prophylactic (Tuchscher *et al.*, 2008). Use of antibodies or serums in a passive immunization can be combined with other agents such as an antibiotic to increase the cure rate of an infection currently in progress or to increase protection against an imminent infection.

**[00141]** Active immunization is administration of the polypeptides, nucleic acids or delivery systems as described herein to a subject.

**[00142]** The components identified in accordance with the teachings of the present invention have a prophylactic and/or therapeutic value such as they can be used to raise an immune response to prevent and/or combat diseases or conditions, and more particularly diseases or conditions related to microbial infections.

**[00143]** The terms "prevent/preventing/prevention" or "treat/treating/treatment" as used herein, refer to eliciting the desired biological response, *i.e.*, a prophylactic and therapeutic effect, respectively in a subject. In accordance with the present invention, the therapeutic effect comprises one or more of a decrease/reduction in the severity, intensity and/or duration of the microbial infection (e.g., staphylococcal infection) or any symptom thereof following administration of the polypeptide, nucleic acid or delivery system (agent/composition of the present invention) of the present invention when compared to its severity, intensity and/or duration in the subject prior to treatment or as compared to that/those in a non-treated control subject having the infection or any symptom thereof. In accordance with the invention, a prophylactic effect may comprise a delay in the onset of the microbial infection (e.g., staphylococcal infection) or any symptom thereof in an asymptomatic subject at risk of experiencing the microbial infection (e.g., staphylococcal infection) or any symptom thereof at a future time; or a decrease/reduction in the severity, intensity and/or duration of a microbial infection (e.g., staphylococcal infection) or any symptom thereof occurring following administration of the agent/composition of the present invention, when compared to the timing of their onset or their severity, intensity and/or duration in a non-treated control subject (*i.e.* asymptomatic subject at risk of experiencing the microbial (e.g., bacterial) infection (e.g., staphylococcal infection) or any symptom thereof); and/or a decrease/reduction in the progression of any pre-existing microbial infection (e.g., staphylococcal infection) or any symptom thereof in a subject following administration of the agent/composition of the present invention when compared to the progression of microbial infection (e.g., staphylococcal infection) or any symptom thereof in a non-treated control subject having such pre-existing microbial infection (e.g., staphylococcal infection) or any symptom thereof. As used herein, in a therapeutic treatment, the agent/composition of the present invention is administered after the onset of the microbial infection (e.g., staphylococcal infection) or any symptom thereof. As used herein, in a prophylactic treatment, the agent/composition of the present invention is administered before the onset of the microbial

infection (e.g., staphylococcal infection) or any symptom thereof or after the onset thereof but before the progression thereof.

**[00144]** As used herein, “decrease” or “reduction” of microbial infection (e.g., staphylococcal infection) or any symptom thereof refers to a reduction in a symptom of at least 10% as compared to a control subject (a subject not treated with the agent/composition present invention), in an embodiment of at least 20% lower, in a further embodiment of at least 30% lower, in a further embodiment of at least 40% lower, in a further embodiment of at least 50% lower, in a further embodiment of at least 60% lower, in a further embodiment of at least 70% lower, in a further embodiment of at least 80% lower, in a further embodiment of at least 90% lower, in a further embodiment of 100% (complete inhibition).

**[00145]** As used herein, the term “symptom” in reference to a staphylococcal infection refers to any staphylococcal infection symptom such as pain, inflammation, fever, vomiting, diarrhea, fatigue muscle aches, anorexia, dehydration, low blood pressure, cellulitis, impetigo, boil and scalded skin syndrome. More particularly, in reference to a staphylococcal IMI, a staphylococcal IMI symptom refers for example to visual abnormalities in milk (e.g., such as a watery appearance, flakes, clots, malodorous, presence of blood), redness of the udder, swelling in the udder, tenderness in the udder, elevated rectal temperature ( $>39.0^{\circ}\text{C}$ ), anorexia, decreased rumen motility and fatigue. An increase in milk somatic cell counts (SCC) is another staphylococcal IMI. Milk somatic cells include white blood cells such as leukocytes or neutrophils as well as epithelial cells. It is generally agreed that a SCC of  $>200,000/\text{mL}$  may represent a staphylococcal IMI symptom or is indicative of a staphylococcal IMI.

### **Dosage**

**[00146]** Toxicity or efficacy of vaccine components to elicit an immune response can be determined by standard procedures in cell cultures or experimental animals. The dose ratio between toxic and immune stimulatory effects can be measured. Components that exhibit large ratios are preferred. While components that exhibit toxic side effects may be used, care should be taken to design a delivery system in order to minimize potential damage to cells and, thereby, reduce side effects.

**[00147]** Data obtained from cell culture assays and laboratory animal studies can be used in formulating a range of dosage for use in large animals and humans. The dosage of such components lies preferably within a range of administered concentrations that include efficacy with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

**[00148]** Any suitable amount of the pharmaceutical composition may be administered to a subject. The dosages will depend on many factors. Typically, the amount of active ingredient contained within a single dose will be an amount that effectively prevents, or treats IMI without inducing significant toxicity. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively raise an immune response in a subject. Moreover, the therapeutically effective amount of the antigens (e.g., fusion construct) of the present invention may require a series of doses. In general, an amount of about 0.01 mg - 500 mg of antigens including the fusion construct per dose, come into consideration. In a specific embodiment, an amount of about 0.1 mg – 1

mg of antigens including the fusion construct per dose, come into consideration. Generally, one, two or three doses of the vaccine may favor optimal development of immunity. The time between two doses may be as short as three or four weeks but it may be preferred to separate the priming dose (first dose) and the booster dose (second dose) by five, six, seven, eight, nine or ten weeks before stimulating the immune system with the booster shot. A subsequent booster shot (a recall shot) may also be optimal to provide a sustainable immunity. This recall could for example occur every half year (6 months), yearly, every two years, every three or every five years.

**[00149]** "Sample" or "biological sample" refers to any solid or liquid sample isolated from a live being. In a particular embodiment, it refers to any solid (e.g., tissue sample) or liquid sample isolated from a mammal, such as milk, a biopsy material (e.g., solid tissue sample), blood (e.g., plasma, serum or whole blood), saliva, synovial fluid, urine, amniotic fluid and cerebrospinal fluid. Such sample may be, for example, fresh, fixed (e.g., formalin-, alcohol- or acetone-fixed), paraffin-embedded or frozen prior to analysis of the infectious agent's expression level.

#### **Patients**

**[00150]** As used herein the term "subject" or "patient" refers to an animal, preferably a mammal such as but not limited to a human, cow (e.g., heifer, multiparous, primiparous, calf), goat, sheep, ewe, ass, horse, pig, chicken, cat, dog, etc. who is the object of treatment, observation or experiment. In a specific embodiment, it is a cow (e.g., at risk of experiencing staphylococcal (e.g., IMI) infection).

**[00151]** As used herein the terms "subject at risk of experiencing a staphylococcal infection (e.g., staphylococcal infection (e.g., IMI) or any symptom thereof at a future time" refers to a mammal (e.g., a cow (e.g., heifer, multiparous, primiparous, calf), goat, sheep) that is used for milk or meat production.

**[00152]** In an embodiment, the above-mentioned mammal is a cow.

#### **Method of detection**

**[00153]** Examples of methods to measure the amount/level of selected proteins/polypeptides include, but are not limited to: Western blot, immunoblot, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoprecipitation, surface plasmon resonance, chemiluminescence, fluorescent polarization, phosphorescence, immunohistochemical analysis, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, microcytometry, microarray, microscopy, flow cytometry, and assays based on a property of the protein including but not limited to DNA binding, ligand binding, interaction with other protein partners or enzymatic activity.

**[00154]** In an embodiment, the amount of the polypeptide/protein within the methods of the present invention is detected using antibodies that are directed specifically against the polypeptide/protein. The term "antibody" as used herein encompasses monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments, so long as they exhibit the desired biological activity or specificity. "Antibody fragments" comprise a portion of a full-length antibody, generally the antigen binding or variable region thereof. Interactions between antibodies and a target polypeptide are detected by radiometric, colorimetric, or fluorometric means. Detection of antigen-antibody complexes may be accomplished by addition of a secondary antibody that

is coupled to a detectable tag, such as for example, an enzyme, fluorophore, or chromophore.

**[00155]** Methods for making antibodies are well known in the art. Polyclonal antibodies can be prepared by immunizing a suitable subject (e.g., rabbit, goat, mouse, or other mammal) with the polypeptide/protein of interest or a fragment thereof as an immunogen. A polypeptide/protein "fragment" "portion" or "segment" is a stretch of amino acid residues of at least about 5, 7, 10, 14, 15, 20, 21 or more amino acids of the polypeptide noted above. The antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized exosomal marker polypeptide or a fragment thereof. At an appropriate time after immunization, e.g., when the antibody titers are highest, antibody-producing cells can be obtained from the animal, usually a mouse, and can be used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256: 495-497, the human B cell hybridoma technique (Kozbor et al. (1983) *Immunol. Today* 4: 72), the EBV-hybridoma technique (Cole et al. (1985) in *Monoclonal Antibodies and Cancer Therapy*, ed. Reisfeld and Sell (Alan R. Liss, Inc., New York, NY), pp. 77-96) or trioma techniques. The technology for producing hybridomas is well known (see generally Coligan et al., eds. (1994) *Current Protocols in Immunology*, John Wiley & Sons, Inc., New York, NY).

**[00156]** Alternatively, to preparing monoclonal antibody-secreting hybridomas, a monoclonal antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with a polypeptide or a fragment thereof to thereby isolate immunoglobulin library members that bind the polypeptide. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System™, Catalog No. 27-9400-01; and the Stratagene SurfZAP™ Phage Display Kit, Catalog No. 240612).

**[00157]** Furthermore, antibodies directed against one or more of the polypeptides/proteins described herein may be obtained from commercial sources.

**[00158]** The use of immobilized antibodies specific for the polypeptides/proteins is also contemplated by the present invention and is well known by one of ordinary skill in the art. The antibodies could be immobilized onto a variety of solid supports, such as magnetic or chromatographic matrix particles, the surface of an assay plate (such as microtiter wells), pieces of a solid substrate material (such as plastic, nylon, paper), and the like. An assay strip could be prepared by coating the antibody or a plurality of antibodies in an array on solid support. This strip could then be dipped into the test sample and then processed quickly through washes and detection steps to generate a measurable signal, such as a colored spot.

**[00159]** The analysis of a plurality (2 or more) of polypeptides/proteins may be carried out separately or simultaneously with one test sample. Several polypeptides/proteins may be combined into one test for efficient processing of a multiple of samples.

**[00160]** The analysis of polypeptides/proteins could be carried out in a variety of physical formats as well. For example, the use of microtiter plates or automation could be used to facilitate the processing of large numbers of test samples. Alternatively, single sample formats could be developed to facilitate immediate treatment and

diagnosis in a timely fashion. Particularly useful physical formats comprise surfaces having a plurality of discrete, addressable locations for the detection of a plurality of different analytes. Such formats include protein microarrays, or "protein chips" (see, e.g., Ng and Ilag, J. Cell Mol. Med. 6: 329-340, 2002) and capillary devices.

**[00161]** In an embodiment, the above-mentioned level of expression is determined by measuring the level of expression of a mRNA transcribed from said one or more genes.

**[00162]** Methods to determine nucleic acid (mRNA) levels are known in the art, and include for example polymerase chain reaction (PCR), reverse transcriptase-PCR (RT-PCR), SAGE, quantitative PCR (q-PCR), Southern blot, Northern blot, sequence analysis, microarray analysis, detection of a reporter gene, or other DNA/RNA hybridization platforms. For RNA expression, preferred methods include, but are not limited to: extraction of cellular mRNA and Northern blotting using labeled probes that hybridize to transcripts encoding all or part of one or more of the nucleic acids encoding the protein/polypeptide of this invention; amplification of mRNA expressed from one or more of the nucleic acids encoding the proteins/polypeptides of this invention using specific primers, polymerase chain reaction (PCR), quantitative PCR (q-PCR), and reverse transcriptase-polymerase chain reaction (RT-PCR), followed by quantitative detection of the product by any of a variety of means; extraction of total RNA from the biological sample, which is then labeled and used to probe cDNAs or oligonucleotides encoding all or part of the nucleic acids encoding the proteins/polypeptides of this invention, arrayed on any of a variety of surfaces.

#### **Kits**

**[00163]** The present invention also encompasses kits comprising the components of the present invention. For example, the kit can comprise one or more components. The components can be packaged in a suitable container and device for administration. The kit can further comprise instructions for using the kit.

**[00164]** The present invention also provides a kit or package comprising reagents useful for administering one or more construct, polypeptide, nucleic acid, vector, host, compositions of the present invention, or a combination of at least two thereof, to a subject in need thereof for treating and/or preventing Staphylococcal IMI. Such kit may further comprise, for example, instructions for the prevention and/or treatment of Staphylococcal IMI, containers, reagents useful for performing the methods. The kit may further include where necessary agents for reducing background interference in a test, agents for increasing signal, software and algorithms for combining and interpolating marker values to produce a prediction of clinical outcome of interest, apparatus for conducting a test, calibration curves and charts, standardization curves and charts, and the like.

#### **MODE(S) FOR CARRYING OUT THE INVENTION**

**[00165]** The present invention is illustrated in further details by the following non-limiting examples.

#### **EXAMPLE 1: Materials and Methods for Vaccine including SACOL0029, SACOL0442, SACOL0720, SACOL1867, SACOL1912 and SACOL2385 (Vaccine #1)**

**[00166]** *Production of the antigens.* Six antigens that are highly expressed during *S. aureus* bovine intramammary infection were selected for inclusion in a first bovine vaccine (Vaccine #1). These antigens are:

SACOL0029 (GenBank™ accession No.: YP\_184940.1) (SEQ ID NO: 5), SACOL0442 (YP\_185332.1) (SEQ ID NO: 29), SACOL0720 (YP\_185601.1) (SEQ ID NO: 11), SACOL1867 (GenBank™ accession No.: YP\_186695.1) (SEQ ID NO: 38), SACOL1912 (GenBank™ accession No.: YP\_186737.1) (SEQ ID NO: 43), and SACOL2385 (GenBank™ accession No.: YP\_187189.1) (SEQ ID NO: 50). His-tagged recombinant proteins of SACOL0029, SACOL1867, SACOL1912, and SACOL2385 were engineered and produced by GenScript, Inc. (Piscataway, NJ). His-tagged recombinant proteins of SACOL0442 and SACOL0720 were engineered and produced using QIA expression technology (pQE30 plasmid) from Qiagen Inc. (Mississauga, ON, Canada), according to the manufacturers' recommendations. See, FIG. 21 I to VI for the his-tagged sequences of the antigens. Examples 2-5 and FIGs. 1-4 relate to this vaccine #1.

**[00167]** *Immunization of dairy cows.* Nineteen healthy multiparous Holstein cows in mid-lactation were housed in a level II biosafety barn at the Dairy and Swine Research and Development Centre of Agriculture and Agri-Food Canada (Sherbrooke, QC). Cows were randomly divided into 2 groups: one group (10 cows) received saline (placebo group); the other group (9 cows) received the vaccine #1 (vaccinated group). The vaccine was composed of 300 µg of each of six antigens (SACOL0029, SACOL0720, SACOL1867, SACOL1912, and SACOL2385) combined with Emulsigen™-D (MVP Technologies, Omaha, NE), CpG ODN 2007 (TCGTCGTTGTCGTTTGTGCGTT (SEQ ID NO: 194), a pathogen-associated molecular pattern (PAMP), VIDO, Saskatoon, SW) and the cationic peptide indolicidin (ILPWKWPWWPWRR (SEQ ID NO: 195), used to induce the cow's immune response, (Chemprep Inc., Miami, FL). Two immunizations were performed 10 weeks apart, subcutaneously in the neck. No adverse side effects were observed. Blood from the caudal vein and milk samples were taken before the first immunization (preimmune serums) and then every two weeks for the detection of total IgG, IgG1 and IgG2. Larger volumes of blood from the jugular vein (150 mL was taken before the first immunization and 14 weeks after the first immunization (i.e., 4 weeks after the second immunization) for peripheral blood mononuclear cells (PBMCs) isolation and analysis of the cellular immune responses.

**[00168]** *Detection of total IgG, IgG1 and IgG2 by ELISA.* Detection of total IgG, IgG1 and IgG2 against each of the antigens in serum and milk was performed as previously described with some modifications (Ster et al., *Vet. Immunol. Immunopathol.* (2010), 136: 311-318). Nunc MaxiSorp™ 96-well plates (Thermo Fisher Scientific Inc., Rochester, NY) were coated with the test antigen (5 µg/mL diluted in carbonate/bicarbonate buffer, Sigma Aldrich, Oakville, ON) and incubated overnight at 37°C. The plates were then saturated with the PBS containing 0.5% gelatin (BD, Franklin Lakes, NJ) for 1 h at 37°C. One hundred microliters of two-fold serial dilutions of the sera in PBS containing 0.5% gelatin and 0.1% Tween™ 20 were loaded into the plates and incubated for 1 h at 37°C. The plates were washed three times with PBS containing 0.1% Tween™ 20. One hundred microliters of horseradish peroxidase (HRP)-conjugated secondary antibody were added to the plate. The secondary antibodies used were a goat anti-bovine IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA), a sheep anti-bovine IgG1 (AbD Serotec, Raleigh, NC) or a sheep anti-bovine IgG2 (AbD Serotec), diluted 1/50,000 1/20,000 and 1/20,000 respectively in PBS containing 0.5% gelatin and 0.1% Tween™ 20. After 1 h of incubation at 37°C followed by 3 washes, peroxidase activity was detected with 3,3',5,5'-tetramethylbenzidine (TMB) reagent (KPL Inc., Gaithersburg, MD) according to the manufacturer's

recommendations.

**[00169]** Detection of total IgG, IgG1 and IgG2 in milk was carried out using the same procedure with few modifications. Milk samples were diluted into PBS containing 0.5% gelatin. The sheep anti-bovine IgG2 was diluted 1/10,000 into PBS containing 0.5% gelatin and 0.1% Tween<sup>TM</sup> 20.

**[00170]** *Evaluation of the cellular immune response.* PBMCs were isolated from jugular vein blood and labelled with carboxyfluorescein diacetate, succinimidyl ester (CFDA-SE; Molecular Probes Inc., Eugene, OR) as previously described (Loiselle et al., *J. Dairy. Sci.* (2009), 92:1900-1912). At the end of the CFDA-SE labelling procedure, the PBMCs were suspended in RPMI medium containing 5% FBS and 1X antibiotic/antimycotic (A5955, Sigma Chemical Aldrich). The PBMCs ( $5 \times 10^6$  cells per well) were stimulated with the mitogen concanavalin A (ConA; positive control; Sigma Aldrich) at a final concentration of 1  $\mu\text{g/mL}$ , or each antigen (5  $\mu\text{g}$  per well) and incubated for 7 days at 37°C. As a negative control, the PBMCs were incubated without any mitogen. Stimulations were performed in duplicate (Ster et al., 2010).

**[00171]** The proliferation of CD4<sup>+</sup> and CD8<sup>+</sup> cells was evaluated after incubation with the different mitogens. The cells were centrifuged at 300 × g for 5 min, suspended in PBS containing 0.5% BSA. The mouse anti-bovine CD8 coupled with Alexa Fluor<sup>TM</sup> 647 (diluted 1/20, AbD Serotec) and the mouse anti-bovine CD4 coupled with rPE (diluted 1/20, AbD Serotec) were then added. After 20 min of incubation on ice, the cells were washed three times with PBS containing 0.5% BSA. The cells were then suspended in PBS with 0.5% formaldehyde. The percentages of the proliferative populations were determined by flow cytometry on a BD FACS Canto II flow cytometer using the BD FACS Diva software.

**[00172]** *Experimental S. aureus in IMI in dairy cows.* Before their use in experimental IMI, the relationship between the absorbance of the bacterial cultures (A600 nm) and CFU was determined. The day of the challenge, a volume of the overnight culture of *S. aureus* in Mueller Hinton broth (MHB; BD) was transferred to 200 mL of fresh MHB to obtain an A600 nm of 0.1 and subsequently grown at 35°C until the A600 nm reached a value corresponding to  $10^8$  CFU/mL in the exponential phase of growth. The strain to be used in this experimental infection (CLJ08-3) had previously been characterized in the co-inventor's lab (Allard et al., *Vet. Microbiol.* (2013) 162: 761- 770). For intramammary infusions, bacteria were routinely diluted in sterile PBS (Sigma Aldrich) to obtain approximately 50 CFU in 3 mL. In this experiment, the inoculum was plated on TSA and found to contain 63 cfu in 3 mL.

**[00173]** Somatic cell count (SCC) determinations and bacterial analysis of aseptic quarter milk samples were carried out prior to experimental IMI to ensure that all cows were free of IMI. Experimental infusion of mammary quarters with bacteria was performed in three (randomly chosen) of the four quarters of each cow after the evening milking according to a procedure previously described (Petitclerc et al., *J. Dairy. Sci.* (2007), 90: 2778-2787) with few modifications. Briefly, before inoculation, teats were scrubbed with gauze soaked in 70% ethanol. Teats were allowed to air-dry before intramammary infusion of 3 mL of bacterial suspension (containing 63 CFU) into three of the four quarters. Immediately after infusion, all quarters were thoroughly massaged and teats were dipped in an iodophore-based teat sanitizer. Disposable gloves were worn throughout the procedure and

disinfected before proceeding to the next animal. All quarters infused with *S. aureus* became infected and all cows showed clinical signs (inflammation, and/or poor milk appearance) of mastitis at some time during the first few days after infusion of *S. aureus*.

**[00174]** *Evaluation of the S. aureus viable counts after experimental infections.* Aseptic milk samples were taken before the morning milking three times a week during the 3 first weeks following the experimental infection and then twice a week for the 2 remaining weeks. After foremilk was discarded and the teats were disinfected with 70% ethanol, a 10-mL milk sample was aseptically collected in a 50-mL sterile vial for each individual quarter. Milk samples were serially diluted and 100  $\mu$ L of each dilution were plated on both tryptic soy agar (Becton Dickinson) and mannitol salt agar plates (Becton Dickinson) for CFU determinations and *S. aureus* identification. Plates were then incubated for 24h at 35°C before the colonies were counted. The dilutions that showed between 30 and 300 colonies were used to calculate the bacterial concentration. Each dilution was plated in duplicate.

**[00175]** *Evaluation of the somatic cell counts.* At the same frequency as for aseptic milk samples, milk was harvested using individual quarter milking units at morning milking and weighed for the determination of quarter milk production. A non-aseptic 50-mL sample was also taken from each quarter milking units for the determination of the SCC by a commercial laboratory (Valacta Inc., Ste-Anne-de-Bellevue, QC, Canada). The milking units were thoroughly washed and disinfected with an iodine-based germicide detergent (K.O. Dyne®, GEA Farm Technologies, Westmoreland, NY) between their uses on each cow. All other materials in contact with milk were disinfected with 70% ethanol.

**[00176]** *Statistical analysis.* Statistical analyses of the experimental infection data were performed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) as repeated measurements. For the analysis of SCC and CFU, data were log<sub>10</sub> transformed prior to analysis. Statistical analysis of the antibody titers and of the correlation between CFU and SCC was performed using GraphPad Prism™ v6.05.

**[00177]** *Ethics statement.* All animal experiments were approved by the Agriculture and Agri-Food Canada local institutional animal care committee and conducted in accordance with the guidelines of the Canadian Council on Animal Care.

#### **EXAMPLE 2: Serum total IgG1 titers following vaccination - vaccine #1**

**[00178]** Recombinant His-tagged antigens for SACOL0029 (GenBank™ accession No.: YP\_184940.1) (SEQ ID NO: 5), SACOL0442 (SEQ ID NO: 29), SACOL0720 (SEQ ID NO: 11), SACOL1867 (GenBank™ accession No.: YP\_186695.1) (SEQ ID NO: 38), SACOL1912 (GenBank™ accession No.: YP\_186737.1) (SEQ ID NO: 43), and SACOL2385 (GenBank™ accession No.: YP\_187189.1) (SEQ ID NO: 50), were prepared and administered to healthy cows as described in Example 1 (*Production of the antigens and Immunization of dairy cows*). Nine dairy cows received the vaccine and 10 cows received saline (placebo). Total serum IgG, IgG1 and IgG2 titers were detected as described in Example 1 (*Detection of total IgG, IgG1 and IgG2 by ELISA*).

**[00179]** As expected, and as shown in FIGs 1B-C, immunization induced an increased production of antigen-specific serum IgG1 and IgG2 for the vaccinated group in comparison to the placebo group. Interestingly, the

IgG2/IgG1 ratio was 1 for SACOL0442 (see FIG. 1D), which is an indication of a balanced Th1/Th2 immune response to this antigen. For the antigens SACOL0029, SACOL0720, SACOL1912 and SACOL2385, the IgG2/IgG1 ratio is significantly lower than the ratio for SACOL0442 which indicated that these antigens induced mostly an IgG1 antibody response via the Th2 pathway.

**EXAMPLE 3: Antigen dependent proliferation of blood CD4+ and CD8+ cells following vaccination-vaccine #1**

**[00180]** Antigen dependent proliferation of blood CD4+ and CD8+ cells from the vaccinated cows (9) and placebo cows (10) was evaluated as described in Example 1 (*Evaluation of the cellular immune response*) four weeks after the second immunization (just before the experimental infection) for each antigen. The results for CD4+ cells are shown in FIG. 2, in which each symbol represents the percentage of CD4+ cells that have proliferated for each cow after a week of incubation with the positive control (ConA) or each antigen. Open circles (○) represent data for the vaccinated cows, black squares (■) represent data for the placebo cows. Horizontal lines represent the medians: dashed lines represent the medians for the vaccinated cows while continuous lines represent the medians for the placebo cows.

**[00181]** The symbol \* shows the statistical differences between the vaccinated and the placebo groups for antigens SACOL0029, SACOL0442, SACOL0720 and SACOL1912 (\*,  $P < 0.05$ ).

**[00182]** In addition, the proliferation of CD8+ cells was similar for the vaccinated and placebo cows for all antigens with the exception of the antigen SACOL0720 for which higher proliferation of the CD8+ cells was observed for the vaccinated cows (data not shown). Induction of CD8+ cells also seemed to be important for the resolution of the infection (Riollet et al., 2001; Burton and Erskine, 2003). The vaccine was able to stimulate both cellular (CD8+) and humoral (CD4+) immune response. The vaccine #1 with its different antigens leads to a balanced immune response.

**EXAMPLE 4: Protection effect of the vaccine as evaluated by following the evolution of somatic cell counts (SCC) - vaccine #1**

**[00183]** Experimental *S. aureus* IMI infection in dairy cows were carried out and evaluated as described in Example 1 (*Experimental S. aureus IMI in dairy cows, Evaluation of the S. aureus viable counts after experimental infections, Evaluation of the somatic cell counts and Statistical analysis*). Four weeks and 4 days after the second immunization, 63 CFU of *S. aureus* were infused into 3 of the 4 quarters of the vaccinated (9) and placebo cows (10) at the evening milking (day 1, arrow in FIG. 3). Aseptic milk samples were taken at morning milking and SCC was determined by Valacta (Ste-Anne-de-Bellevue, QC). The results are shown FIG. 3, in which open circles (○) and the dashed line represent data for the vaccinated cows, while the black squares (■) and the continuous line represent data for the placebo cows. Each open circle represents the mean of SCC for all the infected quarters of the vaccinated cows (27) while each square represents the mean of SCC for all the infected quarters of the placebo cows (30 quarters).

**[00184]** Over the challenge period, SCC in milk were found to be significantly lower for the vaccinated cows than for the placebo cows (\*\*\*;  $P < 0.001$ ), indicating less inflammation and a better control of the

infection in the vaccinated cows.

**EXAMPLE 5: Correlation between SCC or the viable counts of *S. aureus* (CFU) relative to serum or milk IgG titers against specific antigens - vaccine #1**

[00185] As shown in FIGs. 4A-B, SCC were positively correlated to *S. aureus* CFU of the challenge period (FIG. 4A,  $r=0.82$ ,  $P<0.0001$ ) and negatively correlated to the serum IgG1 titer against SACOL0442 measured prior to the infection (FIG. 4B,  $r=-0.49$ ,  $P<0.05$ ). Vaccination thus had reduced this criterium of inflammation induced by the challenge. A similar analysis was performed with the samples collected at day 10. The same correlations were observed as previously obtained but at this particular time point SCC and *S. aureus* CFU also correlated to the milk IgG2 titer against SACOL0029 (Fig 4C,  $r=-0.48$ ,  $P<0.05$  and  $r=-0.58$ ,  $P<0.05$ , respectively). These results show that more than one antigen is involved in the immune response against the infection.

**EXAMPLE 6: Materials and methods for vaccine including SACOL0442, SACOL0720, and a fusion between SACOL1867 and SACOL0029 - vaccine #2**

[00186] *Production of the antigens.* Four antigens that are highly expressed during *S. aureus* bovine intramammary infection were selected for inclusion in vaccine #2. The antigens are polypeptides encoded by: SACOL0029 (GenBank™ accession No.: YP\_184940.1) (SEQ ID NO: 5), SACOL1867 (GenBank™ accession No.: YP\_186695.1) (SEQ ID NO: 38), SACOL0442 (SEQ ID NO: 29), and SACOL0720 (SEQ ID NO: 11). The SACOL0029 and SACOL1867 antigens were included in the form of a fusion. His-tagged recombinant proteins of SACOL0720 and SACOL0029-1867 were engineered and produced by GenScript, Inc. (Piscataway, NJ). A his-tagged recombinant protein of SACOL0442 was engineered and produced using QIA expression technology (pQE30 plasmid) from Qiagen Inc. (Mississauga, ON, Canada), according to the manufacturers' recommendations. (see FIGs. 21D-E and I, and items II, III and VII for SACOL0720, SACOL0442, and SACOL0029-1867 his-tagged sequences). The surface protein ClfA (SEQ ID NO: 184), was also additionally produced by using the QIA expression vector by cloning the *clfA* gene from *S. aureus* ATCC 25904. The latter recombinant protein was not part of the vaccine composition but was used in ELISA assays to determine IgG titers of sera against other *S. aureus* proteins such as ClfA.

[00187] The vaccine was composed of 300 µg of each of 3 antigens (SACOL0442 and SACOL0720 as defined in Example 1 and the fusion SACOL0029-1867) and with Emulsigen™-D (MVP Technologies, Omaha, NE), CpG ODN 2007 (i.e. TCGTCGTTGTCTTTTGTCTT (SEQ ID NO: 194) (IDT, Coralville, IA)), and indolicidin (ILPWKWPWWPWRR (SEQ ID NO: 195, GenScript, Piscataway, NJ, Chemprep Inc., Miami, FL) (vaccine #2).

[00188] *Immunization of dairy cows.* Eleven healthy multiparous Holstein cows in mid-lactation were housed in a level II biosafety barn at the Dairy and Swine Research and Development Centre of Agriculture and Agri-Food Canada (Sherbrooke, QC). Cows received the vaccine #2 (vaccinated group). Two immunizations were performed 10 weeks apart, subcutaneously in the neck. No adverse side effects were observed. Blood from the caudal vein and milk samples were taken before the first immunization (preimmune serums) and then every week for the detection of total IgG.

[00189] *Detection of total IgG by ELISA.* Detection of total IgG against each of the antigens in serum was

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performed as previously described with some modifications (Ster et al., *Vet. Immunol. Immunopathol.* (2010), 136: 311-318). Nunc MaxiSorp™ 96-well plates (Thermo Fisher Scientific Inc., Rochester, NY) were coated with the test antigen (5 µg/mL diluted in carbonate/bicarbonate buffer, Sigma Aldrich, Oakville, ON) and incubated overnight at 37°C. The plates were then saturated with the PBS containing 0.5% gelatin (BD, Franklin Lakes, NJ) for 1 h at 37°C. One hundred microliters of two-fold serial dilutions of the sera in PBS containing 0.5% gelatin and 0.1% Tween™ 20 were loaded into the plates and incubated for 1 h at 37°C. The plates were washed three times with PBS containing 0.1% Tween™ 20. One hundred microliters of horseradish peroxidase (HRP)-conjugated secondary antibody were added to the plate. The secondary antibodies used were a goat anti-bovine IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) diluted 1/1000,000 in PBS containing 0.5% gelatin and 0.1% Tween™ 20. After 1 h of incubation at 37°C followed by 3 washes, peroxidase activity was detected with 3,3',5,5'-tetramethylbenzidine (TMB) reagent (KPL Inc., Gaithersburg, MD) according to the manufacturer's recommendations.

**EXAMPLE 7: The fusion of antigens induces high antibody titers - vaccine #2**

[00190] FIG. 5 shows serum total IgG titers for the vaccinated cows for each antigen of the vaccine (including the fused antigens SACOL0029 and SACOL1867 (labeled SACOL0029-1867 on FIG. 5). Each open circle represents the titer four weeks after the second immunization for each cow (just before the experimental infection) whereas each black diamond represents the preimmune titer. Horizontal lines represent the medians: solid line for the preimmune serums, dotted line for the samples taken four weeks after immunization. Titers for the vaccinated cows are higher than the titers of the preimmune serums (\*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$  for the other antigens tested).

[00191] FIG. 5 thus shows that the vaccine composed of three separate antigens, including a fusion peptide, induces a strong immune response in the cows. Furthermore, FIG. 5 surprisingly shows that the fused antigens SACOL0029 and SACOL1867 (fusion SACOL0029-SACOL1867) raised antibody titers that were above those raised by each of the antigen alone (Compare FIG. 1A vs. FIG. 5) and that such fused antigens provide an additional benefit to the vaccine.

[00192] More particularly, when administered individually in a vaccine, the titers of immune cows against SACOL0029 and SACOL1867 reached 3200 and 51200, respectively (FIG. 1A), whereas when these antigens were administered as a fusion, the titers of immune cows reached 12800 and 409600, respectively (FIG. 5), showing that the fusion create an unexpected synergy in the immune response.

**EXAMPLE 8: Materials and methods for vaccine comprising SACOL0029, SACOL1867, and a fusion between SACOL1867 and SACOL0029 - vaccine #3**

[00193] *Production of the antigens.* Three antigens derived from two genes that are highly expressed during *S. aureus* bovine intramammary infection were selected for inclusion in a vaccine. These antigens are: SACOL0029 (GenBank™ accession No.: YP\_184940.1) (SEQ ID NO: 5), SACOL1867 (GenBank™ accession No.: YP\_186695.1) (SEQ ID NO: 38) and a fusion between SACOL1867 and SACOL0029 (GenBank™ accession

No.: YP\_184940.1) (SEQ ID NO: 5). His-tagged recombinant proteins of SACOL0029, SACOL1867 and SACOL0029-1867 were engineered and produced by GenScript, Inc. (Piscataway, NJ). (see FIGs. 21A, F and I, items I, IV and VII for SACOL0029, SACOL1867, and SACOL0029-1867 his-tagged sequences).

**[00194]** *Immunization of mice.* The immunogenic properties of recombinant *S. aureus* proteins encoded by the SACOL0029, SACOL1867 genes and a fusion of SACOL0029 and SACOL1867 were evaluated in mice. Four groups of mice received the exact equimolar quantity of proteins, either in a monovalent form (SACOL0029 or SACOL1867) or in a multivalent form (the fusion SACOL0029-1867 or SACOL0029 together with SACOL1867 in combination), were compared.

**[00195]** In brief, the theoretical molecular weight of each amino acid sequence corresponding to the entire fusion or to the SACOL0029 or SACOL1867 portion of the fusion were calculated using the ExPASy™ Bioinformatic resource portal ([http://web.expasy.org/cgi-bin/compute\\_pi/pi\\_tool](http://web.expasy.org/cgi-bin/compute_pi/pi_tool)).

**[00196]** Five micrograms of the fusion were administered to one group of mice. The corresponding molar quantity of 5 µg of the SACOL0029-1867 fusion was determined to be 168.55 pmol, in regard to its theoretical molecular weight. An amount of 1.15 µg and 3.69 µg of SACOL0029 and SACOL1867, respectively was administered in two other groups of mice in order to provide 168.55 pmol of each antigen, respectively. The last group of mice received the combination of the two individual antigens (168.55 pmol of each).

**[00197]** For the preparation of the immunization doses, SACOL0029, SACOL1867 and the SACOL0029-1867 fusion polypeptides were individually mixed and suspended in PBS to obtain the final equimolar quantity of each antigenic dose in a volume of 100 µl. Twenty CD-1 female mice were randomly divided into 4 groups: group A (5 mice) received 5 µg of the SACOL0029-1867 fusion protein (**Fusion**); group B (5 mice) received 1.15 µg of SACOL0029 and 3.69 µg of SACOL1867 (**Combination**); group C (5 mice) received 1.15 µg of SACOL0029 (**0029**) and group D received 3.69 µg of SACOL1867 (**1867**). The CD-1 mice were immunized by two subcutaneous injections in the neck two weeks apart. No adverse side effects were observed during the totality of the experimental immunization period. Blood samples were taken just before the first priming injection (preimmune serums) and ten days after the boost immunization (immune serums). The blood aliquots were allowed to clot at room temperature for an hour, centrifuged at 10,000 g for 10 min at 4°C. The sera were harvested and kept at -20°C until subsequent analysis.

**[00198]** *Detection of total IgG by ELISA.* Detection of serum total IgG against SACOL0029 and SACOL1867 recombinant proteins was performed as previously described with some modifications (Ster et al., *Vet. Immunol. Immunopathol.* (2010), 136: 311-318). Nunc MaxiSorp™ 96-well plates (Thermo Fisher Scientific Inc., Rochester, NY) were coated with 75 µl of each of the test antigen (6.67 µg/mL diluted in carbonate/bicarbonate buffer, Sigma Aldrich, Oakville, ON) and incubated overnight at room temperature. The plates were then saturated with PBS containing 5% skim milk powder for 1 h at 37°C. One hundred microliters of four-fold serial dilutions of the sera in PBS containing 3% milk and 0.025% Tween™ 20 were loaded into the plates and incubated for 1 h at 37°C. The plates were washed three times with PBS containing 0.05% Tween™ 20. One hundred microliters of horseradish peroxidase (HRP)-conjugated secondary antibody were then added to the plate. The secondary

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antibody used was a commercial goat anti-mouse IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA), diluted 1/5000 in PBS containing 3% milk and 0.025% Tween™ 20. After 1 h of incubation at 37°C followed by 3 washes, peroxidase activity was detected with the addition of one hundred microliters of 3,3',5,5'-tetramethylbenzidine (TMB) reagent (KPL Inc., Gaithersburg, MD, according to the manufacturer's recommendations.

**[00199]** *Statistical analysis* Statistical analysis of the antibody titers and of the correlation was performed using GraphPad Prism™ v6.05.

**EXAMPLE 9: The fusion of antigens induces significantly higher antibody titers compared to monovalent antigens or a combination of antigens - vaccine #3**

**[00200]** FIG. 6 shows that an antigen (SACOL1867) included in a fusion polypeptide (i.e., the SACOL0029-1867 fusion protein) can induce a strong and specific antibody immune response against that specific antigen (SACOL1867), and, more importantly, that this response can be significantly higher than that obtained with a monovalent form of this antigen (SACOL1867 administered alone) or a multivalent combination of individual polypeptides that are part of the fusion (combination of SACOL1867 plus SACOL0029). Thus, in addition to the advantage of generating an immune response against multiple polypeptidic targets, such fused antigens also provide the additional benefit of greatly improving the antibody titers against those targets.

**EXAMPLE 10: Materials and methods for vaccines including SACOL0720 fragment(s) and/or SACOL442 fragment(s) – vaccines #4-6**

**[00201]** *Production of the antigens.* Peptides and amino acid fragments of 15 to 50 amino acids in length and derived from sequences SACOL0442 and/or SACOL0720 were selected based on the presence of B-cell epitopes. Fusions of peptide epitopes were also designed in which an amino acid linker (for example, EAAAKEAAK (SEQ ID NO: 62), or ERKYK (SEQ ID NO: 61) or KDYERKYKKHIVS (SEQ ID NO: 196)) joined the various epitopes. Peptides and amino acid fragments were synthesized by Biomatik, Inc. (Cambridge, ON). Upon receipt, lyophilised peptides and amino acid fragments were suspended in sterile water at a concentration of 5 mg/mL and stored at -80 °C until day of use.

**[00202]** *Immunization of mice.* Peptides and amino acid fragments were used as antigens for immunization of mice. For the preparation of the immunization doses, each peptide and amino acid fragment or a combination of such were mixed and suspended in PBS containing 20 % of the EMULSIGEN®-D oil-in-water emulsion adjuvant to obtain a final dose of 100 µg of polypeptide per dose, unless otherwise specified. CD-1 female mice were randomly divided into different groups of 3 to 4 animals. Mice were immunized by two subcutaneous injections in the neck two weeks apart. No adverse side effects were observed during the totality of the experimental period. Blood samples were taken just before the first priming injection (preimmune serums) and ten days after the boost immunization (immune serums). The blood aliquots were allowed to clot at room temperature for an hour, and then centrifuged at 10,000 g for 10 min at 4°C. The sera were harvested and kept at -20°C until subsequent analysis.

**[00203]** *Detection of total IgG by ELISA.* Detection of serum total IgG, against specific amino acid sequences

found in the antigens used for the immunization of mice, was performed as previously described with some modifications (Ster et al., *Vet. Immunol. Immunopathol.* (2010), 136: 311-318). Nunc MaxiSorp™ 96-well plates (Thermo Fisher Scientific Inc., Rochester, NY) were coated with 100 µl of each of the target amino acid sequences diluted at a final concentration of 5 µg/mL in carbonate/bicarbonate buffer (Sigma Aldrich, Oakville, ON) and incubated overnight at room temperature. The plates were then saturated with PBS containing 5% skim milk powder for 1 h at 37°C. One hundred microliters of four-fold or two-fold serial dilutions of the sera in PBS containing 1% milk and 0.025% Tween™ 20 were loaded into the plates and incubated for 1 h at 37°C. The plates were then washed three times with PBS containing 0.05% Tween™ 20. One hundred microliters of horseradish peroxidase (HRP)-conjugated secondary antibody were then added to the plate. The secondary antibody used was a goat anti-mouse IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA), diluted 1/5000 in PBS containing 1% milk and 0.025% Tween™ 20. After 1 h of incubation at 37°C followed by 3 washes with PBS Tween™ 20 and a final wash with PBS, peroxidase activity was detected with 3,3',5,5'-tetramethylbenzidine (TMB) reagent (KPL Inc., Gaithersburg, MD) according to the manufacturer's recommendations.

**[00204]** *Statistical analysis.* Statistical analysis of the antibody titers and optical densities was performed using GraphPad Prism™ v6.05.

**EXAMPLE 11: Immune response against a fusion of peptides that includes epitopes encoded from sequences SACOL0442 and SACOL0720 - vaccine #4**

**[00205]** A fusion of peptide epitopes encoded from SACOL0442 and SACOL0720 was used to vaccinate mice (n = 4). The sequence of the fusion of peptides was **KDGGKYTLESHKELQ***EAAAKEAAAK***KDINKIYFMTD****VDLGGPTFVLND** (SEQ ID NO: 3) (vaccine #4), where the linker is italicized and the different epitopes are identified in bold characters. The epitopes were KDGGKYTLESHKELQ (SEQ ID NO: 1) encoded from SACOL0442, KDINKIYFMTDVL (SEQ ID NO: 23) encoded from SACOL0720, and DVDLGGPTFVLND (SEQ ID NO: 24) also encoded from SACOL0720. The IgG antibodies from the sera harvested from the animals were able to bind amino acid fragments comprising B-cell epitopes from either SACOL0442 (i.e. KDGGKYTLESHKELQ (SEQ ID NO: 1)) and/or SACOL0720 (i.e. QFGFDLKHKKDALA (SEQ ID NO: 21); KDINKIYFMTDVL (SEQ ID NO: 23), DVDLGGPTFVLND (SEQ ID NO: 24)) in ELISA assays with antibody titers of 1/6400 or higher. The fusion of peptides used for immunization and the amino acid fragments or polypeptides used as antibody targets in ELISA assays are shown in Table III below. In this table, the epitopes are in bold and the linker sequence is italicized.

Table III- Polypeptide vaccine and antibody response targets

**Fusion of peptides used for vaccination**

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**KDGGKYTLESHKELQ***EAAAKEAAAK***KDINKIYFMTD****VDLGGPTFVLND** (SEQ ID NO: 3)

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**Peptides and polypeptides targets bound by IgG from vaccinated mice in an ELISA assay**

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**KDGGKYTLESHKELQ***EAAAKEAAAK***KDINKIYFMTD****VDLGGPTFVLND** (SEQ ID NO: 3) (fusion of

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peptides);

GEHLPKGNIVINTKDGGKYTLESHKELQKDRENVKINTAD (SEQ ID NO: 2) (fragment encoded by SACOL0442);

KDINKIYFMTDVLGGPTFVLNDKDYERKYKKHIVSQFGFDLKHKKDALA (SEQ ID NO: 27) (variant comprising fragments encoded by SACOL0720)

**SACOL0442** (SEQ ID NO: 55) (i.e. polyhistidine version shown in FIG. 21E, item II);

**SACOL0720** (SEQ ID NO: 25) (i.e. polyhistidine version shown in FIG. 21D, item III);

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**[00206]** All antibody targets shown above were bound in an ELISA assay by IgG from mice vaccinated with the fusion antigen above.

**[00207]** This demonstrates that a fusion of peptide epitopes encoded by both SACOL0442 and SACOL0720 can be used to immunize and elicit an immune response in a mammal. The obtained immune response includes the production of antibodies that recognize SACOL0442 or SACOL0720, amino acid fragments or variants encoded from either SACOL0442 or SACOL0720.

**EXAMPLE 12: A fusion of multiple epitopes used as an antigen in immunizations significantly enhances the immune response against a single epitope – vaccine #4**

**[00208]** A fusion of peptide epitopes encoded from SACOL0442 and SACOL0720 was used to vaccinate mice (n = 4). The sequence of the fusion of peptides was **KDGGKYTLESHKELQ***EEAAAKEAAAK***KDINKIYFMTDVLGGPTFVLND** (SEQ ID NO: 3), where the linker is italicized and the different epitopes are identified in bold characters. The epitopes were KDGGKYTLESHKELQ (SEQ ID NO: 1) encoded from SACOL0442, KDINKIYFMTDVL (SEQ ID NO: 23) encoded from SACOL0720, and DVLGGPTFVLND (SEQ ID NO: 24) also encoded from SACOL0720. Another group of mice (n = 4) was immunized with the single peptide epitope KDGGKYTLESHKELQ (SEQ ID NO: 1), encoded from SACOL0442.

**[00209]** Sera were collected from animals and tested for the presence of IgG antibodies directed toward an amino acid fragment encoded from SACOL0442 (GEHLPKGNIVINTKDGGKYTLESHKELQKDRENVKINTAD) (SEQ ID NO: 2), which contains the peptide epitope KDGGKYTLESHKELQ (SEQ ID NO: 1).

**[00210]** As shown on FIG. 7, immunization with the fusion of three peptide epitopes (one encoded from SACOL0442 and two encoded from SACOL0720) significantly increased the antibody production against an amino acid fragment encoded from SACOL0442 (GEHLPKGNIVINTKDGGKYTLESHKELQKDRENVKINTAD) (SEQ ID NO: 2), which contains the peptide epitope KDGGKYTLESHKELQ (SEQ ID NO: 1), compared to the antibody level obtained when only using the peptide epitope KDGGKYTLESHKELQ (SEQ ID NO: 1) as antigen for immunization.

**EXAMPLE 13: Immune response against a polypeptide fragment of 50 amino acids encoded a variant of SACOL0720 -vaccine #5**

**[00211]** A group of mice (n = 3) was vaccinated with a 50-amino acid peptide fragment

(**KDINKIYFMTDVLGGPTFVLNDKDYERKYKKHIVSQFGFDLKHKKDALA** (SEQ ID NO: 27)) (vaccine #5) containing B-cell epitopes (bold characters) encoded from the sequence SACOL0720, more specifically epitopes KDINKIYFMTDVL (SEQ ID NO: 23), DVDLGGPTFVLND (SEQ ID NO: 24) and QFGFDLKHKKDALA (SEQ ID NO: 21). The overall sequence of **KDINKIYFMTDVLGGPTFVLNDKDYERKYKKHIVSQFGFDLKHKKDALA** (SEQ ID NO: 27) vary from the native sequence of SACOL0720 by four amino acids in the region linking the epitopes DVDLGGPTFVLND (SEQ ID NO: 24) and QFGFDLKHKKDALA (SEQ ID NO: 21). Vaccine #5 can thus be considered being a variant fragment of SACOL0720 or a fusion of epitopes from SACOL0720, which are spaced by linker ERKYK (SEQ ID NO: 61).

[00212] Sera were tested for the presence of IgG antibodies directed toward a fragment of the native protein encoded by SACOL0720 (SEQ ID NO: 25) (i.e. polyhistidine version shown in FIG. 21D, item II). Both mice vaccinated with the peptide fragment corresponding to the variant sequence of amino acids (or fusion SACOL0720-720) produced antibodies that recognized epitopes in the original sequence of amino acids in an ELISA assay with titers of 1/6400 or higher.

[00213] This demonstrates that an amino acid fragment that comprises epitopes encoded from sequence SACOL0720 can elicit an immune response in a mammal. This also further demonstrates that a variant of the native sequence has the capacity to stimulate the immune system against the original fragment sequence containing B-cell epitopes.

**EXAMPLE 14 : Immune response against a combination of fusions (peptide fusion 0442-0720 and polypeptide fusion 0029-1867) - vaccine #6**

[00214] A fusion of peptide epitopes encoded from SACOL0442 and SACOL0720 (see sequence in FIG. 21I, Item VII- fusions) was combined to a polypeptide fusion containing sequences of SACOL0029 and SACOL1867 (see sequence in FIG. 21I, Item VII fusions) and was used to vaccinate mice (vaccine #6).

[00215] For the preparation of the immunization doses, the peptide fusion 0442-0720 and the polypeptide fusion 1867-0029 were mixed and suspended in PBS containing 20 % of the EMULSIGEN®-D oil-in-water emulsion adjuvant to obtain a final dose of 100 µg and 5 µg per dose of the peptide fusion (0442-0720) and the polypeptide fusion (0029-1867), respectively. CD-1 female mice (n = 3) were immunized by three subcutaneous injections in the neck. The first two injections were made one week apart and the third injection 3 weeks after the second one. No adverse side effects were observed during the totality of the experimental period. Blood samples were taken just before the first priming injection (preimmune serums) and fourteen days after the last boost immunization (immune serums). The blood aliquots were allowed to clot at room temperature for an hour, and then centrifuged at 10,000 g for 10 min at 4°C. The sera were harvested and kept at -20°C until subsequent analysis.

[00216] The IgG antibodies from the sera harvested from the animals were able to bind amino acid fragments comprising epitopes from either SACOL0442 or SACOL0720 or to polypeptide SACOL0029 or SACOL1867 in ELISA assays with antibody titers of 1/6400 or higher. The fusion of peptides and polypeptides used for immunization and the polypeptides or amino acid fragments used as antibody targets in ELISA assays are shown

in the Table IV below.

**Table IV- Mixed polypeptide fusion vaccine and antibody response targets**

A mixture of the fusion of peptides 0442-0720 and polypeptide fusion 0029-1867 was used for vaccination (vaccine #6)

The epitopes are in bold and the linker sequence is italicized

0442-0720: **KDGGKYTLESHKELQ***EAAAKEAAAK***KDINKIYFMTD****VDLGGPTFVLND** (SEQ ID NO: 3)

0029-1867: SACOL0029-*GGGSGGGSGGGGS*-SACOL1867 (SEQ ID NO: 55)

Peptides and polypeptides bound by IgG from vaccinated mice in an ELISA assay

**GEHLPKGNIVINTKDGGKYTLESHKELQ****KDRENVKINTAD** (fragment encoded by SACOL0442) (SEQ ID NO: 2)  
(see sequence in FIG. 21I, Item VII- fusions);

**KDINKIYFMTD****VDLGGPTFVLND****KDYERKYKKHIVSQFGFDLKHKKDALA** (fragment encoded by SACOL0720)  
(SEQ ID NO: 27) (see sequence in FIG. 21I, Item VII- fusions);

SACOL1867 (SEQ ID NO: 40) (see his-tagged sequence in FIG. 21F, Item IV);

SACOL0029 (SEQ ID NO: 8) (see his-tagged sequence in FIG. 21A, Item I);

**[00217]** This demonstrates that a combination of fusions (e.g., peptide fusion 0442-0720 mixed with the polypeptide fusion 0029-1867) can be used to immunize and elicit an immune response in a mammal. The obtained immune response includes the production of antibodies that recognize amino acid sequences encoded from either SACOL0442 or SACOL0720 or SACOL0029 or SACOL1867.

**EXAMPLE 15: Materials and methods for attenuated live mutant**

**[00218]** *Bacterial strains and growth conditions.* Strains used in Examples 15-25 are listed in Table V. *S. aureus* ATCC 29213 and its isogenic mutant  $\Delta 720$  were previously described (Allard *et al.* 2013). Except otherwise stated, *S. aureus* strains were grown in tryptic soy broth (TSB) and agar (TSA) (BD, ON, Canada), and *Escherichia coli* DH5 $\alpha$  were grown in LB and LBA medium (BD). Whenever required, ampicillin (100  $\mu$ g/ml) (Sigma, Oakville, Ontario, Canada), chloramphenicol (20  $\mu$ g/ml) (ICN Biomedicals, Irvine, CA), and erythromycin (10  $\mu$ g/ml) (Sigma) were added to agar plates. For the immunological tests, four different bovine mastitis isolates were selected corresponding to some of the predominant *S. aureus spa* types found in Canadian dairy herds and elsewhere in the world (Veh *et al.*, 2015; Mitra *et al.*, 2013). Strain SHY97-3906 (*spa* t529) was isolated from a case of clinical bovine mastitis that occurred during the lactation period, and CLJ08-3 (*spa* t359) was originally isolated from a cow with persistent mastitis at dry-off (Allard *et al.*, 2013). Strains Sa3151 (*spa* t13401) and Sa3181 (*spa* t267) were obtained from the Canadian Bovine Mastitis and Milk Quality Research Network (CBMMQRN) Mastitis Pathogen Culture Collection (Université de Montréal, Faculté de médecine vétérinaire, St-Hyacinthe, QC, Canada), and were isolated from cases of subclinical intramammary infections.

**Table V- Strains and plasmids used in Examples 15-25**

| Strain or plasmid | Relevant details | Source or reference |
|-------------------|------------------|---------------------|
|-------------------|------------------|---------------------|

| Strains                          |                                                                                            |                                                         |
|----------------------------------|--------------------------------------------------------------------------------------------|---------------------------------------------------------|
| <i>S. aureus</i>                 |                                                                                            |                                                         |
| RN4220                           | Derivative of 8325-4, acceptor of foreign DNA, r-                                          | Kreiswirth <i>et al.</i> (1983)                         |
| ATCC29213                        | Wild Type, SACOL0720 ( <i>vraG</i> ) positive, normal phenotype                            | American Type Culture Collection                        |
| $\Delta 720$                     | SACOL0720 ( <i>vraG</i> ) transposon insertion isogenic mutant of ATCC29213                | Allard <i>et al.</i> (2013)                             |
| $\Delta hemB$                    | <i>hemB::EM<sup>r</sup></i> ; isogenic mutant of ATCC29213, SCV phenotype                  | As described herein                                     |
| $\Delta 720\Delta hemB$          | <i>hemB::EM<sup>r</sup></i> ; isogenic mutant of $\Delta 720$ , SCV phenotype              | As described herein                                     |
| <i>E. coli</i>                   |                                                                                            |                                                         |
| SHY97-3906<br>( <i>spa</i> t529) |                                                                                            | isolated from a case of clinical bovine mastitis        |
| CLJ08-3 ( <i>spa</i> t359)       |                                                                                            | isolated from a cow with persistent mastitis at dry-off |
| Sa3151 ( <i>spa</i> t13401)      |                                                                                            | CBMMQRN) Mastitis Pathogen Culture Collection           |
| Sa3181 ( <i>spa</i> t267)        |                                                                                            | CBMMQRN) Mastitis Pathogen Culture Collection           |
| DH5 $\alpha$                     | <i>lacZDM15 hsdR17 recA1 endA1 gyrA96 thi-1 relA1</i>                                      | Invitrogen (ON, Canada)                                 |
| Plasmids                         |                                                                                            |                                                         |
| pBT2                             | Shuttle vector, temperature-sensitive, AprCmr                                              | Brückner (1997)                                         |
| PBT-E                            | pBT2 derivative, inserted <i>Erma</i> cassette                                             | As described herein                                     |
| pBT-E <i>hemB</i>                | pBT2 derivative, for <i>hemB</i> deletion; Ap <sup>r</sup> Cm <sup>r</sup> Em <sup>r</sup> | As described herein                                     |

**[00219]** *Cell culture conditions.* An established bovine mammary epithelial cell (BMEC) line, MAC-T (Huynh *et al.*, 1991), was used as a cell culture model of infection. The MAC-T cells were routinely cultured and maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum (FBS), supplemented with 5 µg/ml insulin (Roche Diagnostics Inc., Laval, Canada) and 1 µg/ml hydrocortisone (Sigma), and incubated at 37°C in a humidified incubator with 5% CO<sub>2</sub>. Cell culture reagents were purchased from Wisent

(St-Bruno, QC, Canada).

**[00220]** *DNA manipulations.* Recommendations from the manufacturers of kits were followed for genomic DNA isolation (Sigma), plasmid DNA isolation (Qiagen, ON, Canada), extraction of DNA fragments from agarose gels (Qiagen) and purification of PCR products and of digested DNA fragments (Qiagen). An additional treatment of 1h with lysostaphin (Sigma) at 200 µg/ml was used to achieve efficient lysis of *S. aureus* cells in genomic and plasmid DNA isolations. Primers (IDT® Integrated DNA Technologies; Coraville, Iowa, USA) were designed to add restriction sites upstream and downstream of the amplified products. PCRs were performed using the Taq DNA Polymerase (NEB, Pickering, ON, Canada) for routine PCR or the Q5 high fidelity DNA Polymerase (NEB) for cloning, and cycling times and temperatures were optimized for each primer pair. Plasmid constructs were generated using *E. coli* DH5α (Invitrogen, Burlington, ON, Canada), restriction enzymes (NEB), and the T4 DNA ligase (NEB). Plasmid constructs were validated by restriction digestion patterns and DNA sequencing before electroporation in *S. aureus* RN4220 (Kreiswirth *et al.*, 1983) and in final host strains. Plasmids used in Examples 15-25 are listed in Table V above.

**[00221]** *Generation of live attenuated *S. aureus* strain Δ720 and ΔhemB.* An isogenic *hemB* mutant of the ATCC 29213 strain was constructed, in which the *hemB* gene was deleted and replaced by the insertion of an *emrA* cassette by homologous recombination. *S. aureus* ATCC 29213 mutant for gene SACOL0720 (Δ720) was generated using the TargeTron™ Gene Knockout System (with the TargeTron™ Vector pNL9164 (Sigma-Aldrich Canada Ltd.) (Chen *et al.*, 2007) for disruption of bacterial genes by insertion of group II introns (fragment size of approx. 2Kb as previously described (Allard *et al.*, 2013) between nucleotide 803 and 804 in *S. aureus* ATCC29213. The manufacturer protocols and recommendations were followed.

**[00222]** *Generation of ΔhemBΔ720.* To achieve a second mutation in gene *hemB* in order to obtain a SCV phenotype in the Δ720 mutant genetic background, another strategy was used: the temperature-sensitive pBT2-*hemB:emrA* (pBT-E:*hemB*) was used in a strategy previously described (Mitchell *et al.*, 2008), with some modifications. Briefly, the pBT-E plasmid was constructed by the insertion of an *ermA* cassette between XbaI and Sall sites of temperature-sensitive shuttle vector pBT2 (Brückner, 1997). The flanking regions of gene *hemB* (SACOL1715) DNA fragments were amplified from *S. aureus* ATCC 29213 and were cloned on both sides of the *ermA* cassette into the plasmid pBT-E. The plasmid was then transferred for propagation into *S. aureus* RN4220 (res-). After bacterial lysis with lysostaphin (200 µg/ml for 1 h at room temperature), plasmid DNA was isolated and used to transform ATCC 29213 and Δ720 by electroporation. For plasmid integration and mutant generation, bacteria were first grown overnight at 30°C with 10 µg/ml of erythromycin and a 1 µg/ml hemin supplementation (Sigma-Aldrich, ON, Canada). Bacteria were then diluted 1:1000 and grown overnight at 42°C with 2.5 µg/ml of erythromycin and 1 µg/ml hemin. This step was repeated twice. Finally, bacteria were diluted 1:1000 and grown overnight at 42°C without antibiotics. Mutants with the inactivated *hemB* gene were selected as resistant to erythromycin and sensitive to chloramphenicol, together with an SCV phenotype that can be complemented (i.e., reversion to the normal growth phenotype) by a 5 µg/ml hemin supplementation on agar plates. The deletion of *hemB* in the ATCC 29213 (i.e., Δ*hemB*) and Δ720 (i.e., Δ*hemB*Δ720) strains was confirmed by PCR (see FIGs. 8A and B).

**[00223]** *Hemin supplementation in broth culture.* To evaluate the capacity of hemin to restore optimal growth kinetics of *S. aureus*  $\Delta hemB$  and the double mutant  $\Delta 720\Delta hemB$ , overnight bacterial cultures were diluted to an  $A_{600\text{ nm}}$  of approximately 0.1 in culture tubes containing fresh BHI supplemented with hemin (Sigma) added at various concentrations. The  $A_{600\text{ nm}}$  of cultures was monitored at different points in time during the incubation period at 35°C (225 rpm).

**[00224]** *S. aureus infection of bovine mammary epithelial cells (BMECs).* MAC-T BMECs were used for the characterization of intracellular infectivity and persistence of ATCC 29213 (WT) and its isogenic mutants. Forty-eight hours before infection,  $1 \times 10^5/\text{ml}$  MAC-T cells were seeded on treated 24-well plates (Corning) to obtain 30 % confluence. Monolayers were grown to confluence under 10%  $\text{CO}_2$  at 37°C. Six hours prior to infection, monolayers were washed with DMEM and incubated with invasion medium (IM) (growth medium without antibiotics containing 1% heat-inactivated FBS). Overnight bacterial cultures were diluted 1:20 in fresh TSB and grown to mid-logarithmic growth phase, then washed with PBS and diluted in IM to a multiplicity of infection of 10. Invasion was achieved by incubating monolayers with bacteria for 3 h. Monolayers were then washed with DMEM and incubated with IM containing 20  $\mu\text{g}/\text{ml}$  lysostaphin to kill extracellular bacteria. The use of lysostaphin to kill extracellular normal and SCV *S. aureus* was previously validated in cell invasion assays (Moisan *et al.*, 2006 and Tuchscher *et al.*, 2011). The treatment was allowed for 30 min to determine CFUs at 3h of infection, or for an additional 12 or 24 h. Then, following extensive washing with Dulbecco's Phosphate-Buffered Saline (DPBS), monolayers were detached with trypsinization and lysed with 0.05% Triton X-100 and PBS was added to obtain a final 1X concentration. The lysate was serially diluted and plated on TSA for CFUs determination.

**[00225]** *BMECs viability and metabolic activity assay.* To determine the cytotoxic damage inflicted by *S. aureus* ATCC 29213 (WT) and its isogenic mutants on MAC-T cells, the MTT cell metabolic activity assay that measures the reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) into an insoluble formazan product in viable cells, was performed. The assay followed the method of Kubica *et al.* (Kubica *et al.*, 2008) with some modifications. Briefly, *S. aureus* infection of cells was achieved as described in the persistence assay, but instead of lysis after 12 h or 24 h, cells were incubated with 100  $\mu\text{l}$  of MTT reagent (5 mg/ml) (Sigma) in DPBS for 2 h at 37 °C. Following this, an acidic solvent solution of 16 % SDS and 40 % PMF, pH 4.7, was added to lyse the cells and solubilize the crystals of formazan overnight. The samples were read using an Epoch microplate reader (Biotek Instruments Inc.) at a wavelength of 570 nm. All assays were performed in triplicate, and control wells with uninfected cells (high viability control) or lysed WT infected cells (bacteria background control; treated with 0.05 % triton X-100 for 10 min before MTT addition) were included to each plate. The level of metabolic activity was calculated using the following formula:

**[00226]** (absorbance of the sample – background control) / high control)  $\times 100$ .

**[00227]** *Virulence in the mouse mastitis model.* The mouse mastitis model of infection is based on that previously described (Brouillette, 2005; Brouillette, 2004). All the experiments performed with mice were approved by the ethics committee on animal experimentation of the Faculté des sciences of the Université de Sherbrooke and were conducted in accordance with the guidelines of the Canadian Council on Animal Care.

Briefly, one hour following removal of 12-14 day-old offspring, lactating CD-1 mice (Charles River Laboratories) were anesthetized with ketamine and xylazine at 87 and 13 mg/kg of body weight, respectively, and mammary glands were inoculated under a binocular. Mammary ducts were exposed by a small cut at the near ends of teats and a 100  $\mu$ L-bacterial suspension containing  $10^2$  CFUs in endotoxin-free phosphate-buffered saline (PBS, Sigma) was injected through the teat canal using a 32-gauge blunt needle. Two glands (fourth on the right [R4] and fourth on the left [L4] from head to tail) were inoculated for each animal. Mammary glands were aseptically harvested at the indicated times, weighed and visually evaluated for inflammation. Bacterial burden was evaluated after mechanical tissue homogenization in PBS, serial dilutions, and plating on agar for CFU determination. In a second experiment, homogenized glands were conserved for protein extraction for myeloperoxidase (MPO) activity enzymatic assays.

**[00228] Mammary gland protein extraction.** Total protein extraction from mammary glands was performed by an optimized method previously described (Pulli *et al.*, 2013), with some modifications. Mammary tissues were homogenized in a buffer containing a final concentration of potassium phosphate of 50 mM, pH 6.0, and hexadecyltrimethylammonium bromide (CTAB) 50 mM (Sigma). The samples were then sonicated, freeze-thawed in liquid nitrogen, and centrifuged at 2000 g for 15 min at 4°C. Finally, the fat layer was removed by aspiration, and supernatants were saved for a final centrifugation of 15 min at 15 000 g, to discard every cellular debris. Supernatants were distributed in aliquots and kept at -80 ° C until use for the enzymatic assays or protein concentration determination as measured by the bicinchoninic acid method (BCA) Protein Assay Kit (Thermo-Scientific).

**[00229] MPO activity assay.** Neutrophil recruitment in mammary tissues was measured by quantification of MPO enzyme activity by the o-dianisidine-H<sub>2</sub>O<sub>2</sub> method, modified for microplates (Bradley, RD. and Rothstein, GPPC., 1982). In a 96-well microplate, 10  $\mu$ L of tissue extraction supernatants were incubated with a solution of o-dianisidine hydrochloride (0.167 mg/mL) (Sigma) and 0.0005% H<sub>2</sub>O<sub>2</sub> (Sigma) in 50 mM CTAB phosphate buffer 50 mM, pH 6.0. The MPO activity was measured kinetically with intervals of 15 s over a period of 5 min in an Epoch microplate reader at 460 nm. A Unit of MPO was considered as the amount of enzyme that degrades 1  $\mu$ mol of H<sub>2</sub>O<sub>2</sub>/min at 25°C, assuming an absorption coefficient of 11.3 mM<sup>-1</sup> cm<sup>-1</sup> at 460 nm for o-dianisidine (Zhang *et al.*, 2004). Results were expressed as units of MPO per g of gland.

**[00230] Mouse immunizations with the live attenuated mutant  $\Delta 720\Delta hemB$ .** The immunogenic properties of the attenuated strain  $\Delta 720\Delta hemB$  administered as a live vaccine were evaluated in mice. In preliminary studies, the mice well tolerated intramuscular and subcutaneous (SC) injections of the attenuated strain. The doses of  $10^6$ ,  $10^7$  and  $10^8$  CFUs and the SC route were selected for subsequent experiments. For the preparation of bacterial inoculum, *S. aureus*  $\Delta 720\Delta hemB$  colonies previously grown on BHIA plates were washed twice in ice cold PBS and suspended in PBS containing 15% glycerol, then aliquoted and kept at -80 °C until subsequent use. The viable bacterial counts in the inoculum preparation was validated by serial dilution plating on BHIA. CD-1 mice were randomly divided into 3 groups: group 1 (n=3) received a dose of  $10^6$  CFUs; group 2 (n=3),  $10^7$  CFUs, and group 3 (n=3),  $10^8$  CFUs. Mice were immunized by two subcutaneous injections of bacteria in PBS (100  $\mu$ L), in the neck, two weeks apart. Blood samples were taken just before the priming injection (preimmune serums) and

ten days after the boost immunization (immune serums). Blood aliquots were allowed to clot at room temperature for an hour and then centrifuged at 10,000 g for 10 min at 4 °C. The serums were collected and kept at -20 °C until subsequent analysis.

**[00231] Preparation of *S. aureus* cell extracts.** Preparation of *S. aureus* whole cell extracts was done as previously described with some modifications (Asli *et al.*, 2016). Briefly, overnight bacterial cultures were diluted 1/1000 in fresh BHI broth, and then incubated at 35 °C (225 rpm) until an A600nm of ~ 0.8 was reached. Bacterial cells were centrifuged and pellets were washed in ice-cold PBS twice and suspended with the addition of 5 ml of PBS per ml of pellet. Bacterial suspensions were first treated with lysostaphin (Sigma) (100 µg/ml of pellet) for 1 h at 37°C, and then 3 µg of protease inhibitor cocktail (Sigma), 8 µg of RNase A (Sigma) and 8 µg of DNase (Qiagen) per ml of pellet were added to the suspension. After 30 min at room temperature, cells were mechanically disrupted by 3 to 4 passages in a SLM Aminco™ French Pressure cell disrupter, and then centrifuged at 12,000 × g and 4 °C for 10 min to remove unbroken cells. Supernatant was collected and total protein concentration was determined as previously described with the BCA Protein Assay Kit.

**[00232] Detection of mouse total IgG by ELISA.** Detection of serum total IgG against the  $\Delta 720\Delta hemB$  vaccination strain and each of the bovine IMI isolates was performed to demonstrate and measure the systemic humoral response generated by the immunization of mice. For target antigens, Nunc MaxiSorp™ 96-well plates (Thermo Fisher Scientific Inc., Rochester, NY) were coated with 100 µl of each of the whole *S. aureus* cell extracts (10 µg/ml diluted in carbonate/bicarbonate buffer, Sigma), and incubated overnight at room temperature. The plates were then saturated with PBS containing 5% skim milk powder for 1 h at 37°C, followed by a second blocking step with an addition of 5% porcine serum to prevent unspecific *S. aureus* protein A interactions. One hundred microliters of two-fold serial dilutions of the sera in the dilution buffer (PBS with 2% milk, 5% porcine serum and 0.025% Tween™ 20) were loaded into the plates and incubated for 1 h at 37°C. Plates were then washed three times with PBS containing 0.05% Tween™ 20, and loaded with 100 µl of horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) diluted 1/5000 in the dilution buffer. After 1 h of incubation at 37°C followed by 3 washes, peroxidase activity was detected with 3,3',5,5'-tetramethylbenzidine (TMB) reagent (KPL Inc., Gaithersburg, MD) according to the manufacturer's recommendations.

**[00233] Statistical analysis.** Statistical analyses were carried out with the GraphPad Prism™ software (v.6.02). Intracellular bacterial CFUs and bacterial CFUs/g of gland (IMI in mice) were transformed in base 10 logarithm values before being used for statistical analyses. Statistical tests used for the analysis of each experiment and significance are specified in the figure legends.

#### **EXAMPLE 16: Construction of strain *S. aureus* ATCC 29213 $\Delta 720$ , $\Delta hemB$ and $\Delta 720\Delta hemB$**

**[00234]** Live attenuated organisms that mimic natural infection stimulate the immune system in a powerful manner, eliciting broad and robust immune responses that produce both serum and mucosal antibodies, and effector and memory T cells which act synergistically to protect against disease (Detmer, 2006; Kollaritsch, 2000; Pasetti, 2011).

[00235] A mutation in gene SACOL0720 was shown to alter the virulence of *S. aureus* in experimental IMI infections in the cow (Allard et al., 2013).

[00236] Further live-attenuated strains were prepared for vaccine purposes based on the phenotypic aspects of *S. aureus* SCVs. SCVs do not generally generate invasive infections (i.e. additional attenuation) and can be internalized in host cells and therefore will stimulate the cell-mediated immune response in addition to the humoral immune response.

[00237] A stable *S. aureus* SCV was first created through the deletion of the *hemB* gene ( $\Delta hemB$ ) (see Example 15, *Generation of live attenuated S. aureus strain  $\Delta 720$  and  $\Delta hemB$* ). Further attenuation of this SCV was then achieved by inactivation of gene SACOL0720 ( $\Delta 720$ ) (see Example 15, *Generation of  $\Delta hemB\Delta 720$* ).

[00238] After infection of MAC-T bovine mammary epithelial cells, the double mutant ( $\Delta 720\Delta hemB$ ) significantly showed lower internalization and cell destruction compared to that seen with  $\Delta hemB$  and  $\Delta 720$ , respectively.

#### **EXAMPLE 17: Strain *S. aureus* $\Delta hemB\Delta 720$ is attenuated in MAC-T cells**

[00239] The infectivity of ATCC 29213 (WT),  $\Delta 720$ ,  $\Delta hemB$  and  $\Delta hemB\Delta 720$  strains were then compared in intracellular persistence assays using MAC-T cells. By comparing the three mutant strains to their isogenic WT parent, distinct effects of mutations in genes *hemB* and SACOL0720 were observed. A short 3-h incubation of bacteria with cell monolayers followed by the addition of lysostaphin to eliminate extracellular bacteria demonstrated high levels of internalization into MAC-T cells for both WT and  $\Delta hemB$  strains, based on the recovery of viable intracellular bacteria (CFUs) (FIGs. 9A and B). The single  $\Delta 720$  mutant however showed significantly less ( $P \leq 0.01$ ) internalization compared to its parental WT strain (FIG. 9A). The reduction in internalization seen in  $\Delta 720$  was even more pronounced when comparing the double mutant  $\Delta hemB\Delta 720$  to  $\Delta hemB$ , with a 10-fold reduction of inoculum recovery in this 3-h internalization assay ( $P \leq 0.001$ , FIG. 9B). This initial reduction of internalized bacterial load was still apparent 12 and 24 h post invasion (PI) for the double mutant strain  $\Delta hemB\Delta 720$  (FIG. 9C), as illustrated by the  $1\text{-log}^{10}$  reduction of CFU/ml at both time points compared to that observed for  $\Delta hemB$  ( $P \leq 0.001$ ). The difference in initial intracellular bacterial loads between the single  $\Delta 720$  mutant and WT strains (FIG. 9A) gradually vanished with longer incubation times (FIG. 9C), as both strains did not well persist in MAC-T cells (FIG. 10). On the opposite, intracellular CFUs recovered for the single  $\Delta hemB$  strain was significantly higher compared to that recovered for the three other strains at 24 h PI (FIG. 9C,  $P \leq 0.001$  against all). Overall and as expected for the SCV phenotype, the  $\Delta hemB$  strain showed a higher intracellular persistence than any other strain over time (FIG. 10). These results suggest that the  $\Delta 720$  mutation mainly reduces the internalization process into MAC-T cells. Results further demonstrate that the  $\Delta hemB\Delta 720$  mutant is still capable of internalization and persistence into BMECs but at a much lower degree than that seen with the single  $\Delta hemB$  mutant.

[00240] The  $\Delta hemB\Delta 720$  and  $\Delta hemB$  SCVs cause low BMEC disruption. As reported above,  $\Delta hemB$  and  $\Delta hemB\Delta 720$  SCV strains showed a greater persistence over time in MAC-T cells, as illustrated by their sustained viability at 12 and 24 h PI in comparison with WT and  $\Delta 720$  strains (FIGs. 9C and 10). The percentage of the

inoculum recovered from cells stayed nearly the same from 0 to 24 h after lysostaphin addition, both for the double and single *hemB* mutants, with a slight increase at 12 h, indicating intracellular growth (FIG. 10). Both strains started to decrease at a slow rate after this time of infection. However, the apparent reduction of intracellular CFUs for the WT and  $\Delta 720$  strains was concomitant with the visual observation of increasing damage to cell monolayers over time, in comparison to that observed with strains of the SCV phenotypes.

**[00241]** *MAC-T cells viability was also evaluated following infection by each of the four strains studied. MAC-T cell viability was evaluated by the MTT method (Kubica et al., 2008). Results show that both SCV strains ( $\Delta hemB$  and  $\Delta hemB\Delta 720$ ) caused significantly less MAC-T killing in this assay in contrast to the WT and  $\Delta 720$  strains. When compared to  $\Delta hemB$ , the WT strain nearly reduced by half the viability of cells at 12 h (FIG. 11A: WT: 25.4 %;  $\Delta hemB$ : 48.4%). This difference was still apparent at 24 h (FIG. 11B: 16.3% vs. 34.5 %, respectively), even if the bacterial load was 10 times higher for the  $\Delta hemB$  mutant (FIG. 9C). The MAC-T cells were more damaged by  $\Delta hemB$  than by the double mutant  $\Delta 720\Delta hemB$  but the difference was only significant at 24 h ( $P \leq 0.01$ ). When compared directly to the WT strain, the double mutant  $\Delta 720\Delta hemB$  sustained epithelial cells viability 2.3 times more at 12 h (FIG. 11A) and 2.7 times more at 24 h (FIG. 11B) (12 and 24 h:  $P \leq 0.0001$ ). Therefore, the greater intracellular persistence of both SCVs strains compared to the WT and  $\Delta 720$  strains over time (FIG. 10) was likely to be attributed to a lower toxicity of the SCVs to MAC-T cells (FIG. 11). Taken together, results from the BMEC infection assays provided evidence of an additive effect of both  $\Delta hemB$  and  $\Delta 720$  mutations for the attenuation of the WT strain.*

#### **EXAMPLE 18: Strain *S. aureus* $\Delta hemB\Delta 720$ is attenuated in a mouse IMI model**

**[00242]** To attest attenuation of  $\Delta hemB\Delta 720$  in an *in vivo* model of infection, the virulence of the double mutant was evaluated and compared to the WT strain in a murine IMI model (Brouillette and Malouin, 2005). For both strains, the exponential phase of infection took place mainly within the first 12 h post-infection, while the maximal bacterial burden was reached at 24 h for the double mutant and 48 h (day 2 [D2]) for the WT strain (FIG. 12). At 24 h, the double mutant showed a reduction of  $1.9 \log^{10}$  in mean CFU/g of gland compared to WT ( $P \leq 0.05$ ). Also after 24 h, the mutant bacterial burden showed a constant decline until complete bacterial clearance was reached at day 12 (shown by the asterisk on FIG. 12). In contrast, the parental WT strain provoked severe invasive infections compared to the mutant, killing 3 of 9 remaining mice at day 2 and 2 of 3 mice at day 7 (FIG. 12; arrows) before glands could be harvested for those groups. Mice surviving the WT infection maintained high viable counts ( $9 \log^{10}$  CFU/g of gland) at day 7, an approximate  $5 \log^{10}$  difference in bacterial burden compared to the double mutant. These results clearly demonstrate a markedly reduced capacity of strain  $\Delta hemB\Delta 720$  to multiply and survive in the mammary gland. The  $\Delta hemB\Delta 720$  double mutant is therefore strongly attenuated in a mouse intramammary infection (IMI) model and is efficiently cleared from mammary glands.

**[00243]** The attenuated strain  $\Delta hemB\Delta 720$  appears ideal for vaccination purposes and for intracellular delivery of antigens. Indeed, the low and temporary internalization of  $\Delta hemB\Delta 720$  should help stimulation of cell-mediated immunity, a component of the immune response that is important for defense against *S. aureus* (Fowler and Proctor, 2014).

**EXAMPLE 19: Inflammatory response to  $\Delta 720\Delta hemB$  and WT strains following IMI**

[00244] To monitor the inflammatory response (immune response) of the mice to infections with WT and mutant strains, neutrophil infiltration in glands was evaluated by the MPO enzymatic activity of total protein extracts of gland homogenates. MPO activity in biological samples has previously been strongly correlated with absolute number of neutrophils (Xia, 1997), and is hence an adequate marker. During the first hours after infection, neutrophil recruitment followed similar profiles for the double mutant and WT infected glands (FIG. 13), with exponential intensification of apparent neutrophil infiltration from 12 h to 24 h post infection coinciding with bacterial growth albeit with a certain delay. The absolute numbers of polymorphonuclear cells in relation with the bacterial load in mammary glands was previously shown to not always peak at the same time (Brouillette, 2005). No significant difference in MPO activity could be observed at 6, 12 and 24 h between glands infected by mutant and WT strains (FIG. 13). This equivalence in apparent neutrophil infiltration did not however correlate with the visual observation of inflammation at 24 h, at which point the WT infection generated extensive redness of infected glands in comparison to the double mutant (photographs of FIG. 14). On the contrary, mutant infected glands were not visually altered on the macroscopic level compared to PBS controls. The disparity between visual assessment of inflammation and neutrophil infiltration results could be attributed to the differences in bacterial loads (FIGs. 9A-C) and the cytotoxic activity of the WT strain (FIG. 11), and could be coherent with the highly invasive and disseminative capacity of the strain via toxins and enzymes expression. Hence, these results indicate that neutrophil recruitment in the glands infected by the mutant strain was equivalent to that seen with the WT strain and that this was sufficient to allow a subsequent decline and clearance of the mutant bacterial loads.

[00245] Lastly, to confirm strain safety, and to assess that this inflammatory response was not consequent to an inadmissible reactogenic strain, MPO activity was monitored in  $\Delta 720\Delta hemB$  infected glands 4 and 12 days after infection. The level of activity was then compared to levels obtained with PBS injected mice. As illustrated in FIG. 15, the apparent neutrophil presence in mutant infected glands was still high 4 days after infection, with MPO activity ranging from 8 to 21 Units/g of gland. Besides, gland involution, the process by which the lactating gland returns to a morphologically near pre-pregnant state, is ordinarily associated with neutrophilic recruitment that allows phagocytosis of apoptotic cells during the remodelling of tissue (Stein, 2007). In the days following infection in this model, mice glands are already in that normal state of modification, as indicated by their rapid shrinking. However, the MPO levels in mutant infected glands went through a substantial decline between day 4 and 12, ( $P \leq 0.01$ ). MPO levels were then considered to be back to a normal level at day 12 showing no significant difference from that obtained with the PBS-injected mice. The inflammatory response of  $\Delta 720\Delta hemB$  infected glands goes back to normal levels with bacterial clearance (FIG. 15).

**EXAMPLE 20: Immunization with  $\Delta 720G\Delta hemB$  generates a strong humoral response against several *S. aureus* bovine intramammary infection isolates.**

[00246] To confirm that immunization with the live  $\Delta 720\Delta hemB$  can indeed generate a strong immune response suitable for its use as a putative live vaccine against *S. aureus* intramammary infections, mice were immunized with different doses of the live vaccine and serum total IgGs were assayed for binding to whole cell extracts of a variety of *S. aureus* bovine isolates. First, doses of  $10^6$ ,  $10^7$  and  $10^8$  CFUs, administered subcutaneously in the

neck, triggered no adverse effect such as modification of mice behavior or signs of inflammation or necrosis at the immunization site throughout the immunization period. Furthermore, immunization using increasing amounts of the live double mutant ATCC 29213  $\Delta 720\Delta hemB$  yielded increasing titers of systemic IgG antibodies against a whole cell extract of its own antigens (FIG. 15B). The titers of the immune sera were significantly higher than those of the preimmune sera, demonstrating specificity of antibody production against *S. aureus* antigens present in the live vaccine. Most importantly, immunization using increasing amounts of  $\Delta 720\Delta hemB$  also yielded a consequential rise of antibody titers against a variety *S. aureus* strains isolated from bovine mastitis, including strains from the major *spa* types found in Canada and elsewhere in the world (FIG. 15C). These results clearly show that (i) immunization with the double mutant can raise an immune response, and that (ii) the strain background (ATCC 29213) share sufficient common features with bovine mastitis strains so that the antibody response also strongly recognizes strains of major *spa* types.

**[00247]** Immunization of mice using subcutaneous injections of live  $\Delta 720\Delta hemB$  raised a strong humoral response as judged by the high titers of total IgG measured against a whole bacterial cell extract. Also, the vaccine strain  $\Delta 720\Delta hemB$  had sufficient common features with bovine mastitis strains so that the antibody response also strongly recognized strains from a variety of common mastitis associated *spa* types.

**[00248]** Although this demonstrated that the double mutant background (ATCC 29213) share many common features with bovine mastitis strains, such a double mutant can be created in any desired genetic background if one wishes, notably in any strain that was isolated from bovine mastitis, such as but not limited to *S. aureus* strain RF122.

**[00249]** These results show that a SCV strain having some residual intracellular capabilities can allow immune cells recruitment without establishing a severe infection. Such an SCV strain may act as a live-attenuated vaccine that adequately stimulates the immune response to combat pathogens with intracellular abilities.

**EXAMPLE 21: Material and methods - SACOL0442, SACOL0720, SACOL0029 and a fusion between SACOL1867 and SACOL0029 + attenuated live bacteria (vaccine #7)**

**[00250]** *Production of the antigens.* The production of antigens was performed as described in Example 6, in the section *production of the antigens* except for the additional presence of the antigen SACOL0029. His-tagged recombinant proteins of SACOL0029 were engineered and produced by GenScript, Inc. (Piscataway, NJ). (see FIGs. 21A, item I, his-tagged sequence).

**[00251]** *Generation of live attenuated *S. aureus* strain  $\Delta 720\Delta hemB$ .* The generation of live attenuated *S. aureus* strain was performed as described in Example 15 (*Generation of  $\Delta hemB\Delta 720$* ).

**[00252]** *Immunization of mice.* The immunogenic properties of recombinant *S. aureus* proteins encoded by the SACOL0442, SACOL0720, SACOL0029 genes and a fusion of SACOL0029 and SACOL1867 genes in combination, or not, with the live attenuated bacterial strain *S. aureus*  $\Delta 720\Delta hemB$  were evaluated in mice. The mice well tolerated a dose of  $10^3$ ,  $10^5$ ,  $10^6$ ,  $10^7$  and  $10^8$  CFU by subcutaneous injections in the neck and intramuscular injections in the thigh. The dose of  $10^5$  and the subcutaneous route were selected for the following

experiments.

**[00253]** For the preparation of bacterial inoculum, *S. aureus*  $\Delta 720\Delta hemB$  colonies previously grown on BHIA plates were washed twice in ice cold PBS and resuspended in PBS containing 15% glycerol, then aliquoted and kept at -80°C until subsequent use. To obtain the final mice immunization dose, corresponding to  $10^5$  CFU of attenuated bacteria, the frozen inoculum bacterial concentration was evaluated by serial dilution plating on BHIA and then was diluted to a final concentration of  $10^5$  CFU/ml in PBS on the day of immunization.

**[00254]** For the preparation of protein doses, SACOL0029, SACOL0442, SACOL0720, and the SACOL0029-1867 fusion polypeptide were mixed and suspended in PBS to obtain a final dose of 5  $\mu$ g each. CD-1 female mice were randomly divided into 3 groups: group 1 (5 mice) received a mixed protein dose (protein Mix); group 2 (5 mice) received an attenuated bacteria ( $\Delta 720\Delta hemB$ ) dose ( $\Delta 720\Delta hemB$ ); group 3 (6 mice) received a combination of mixed proteins and attenuated bacteria (combination). CD-1 female mice were immunized by two subcutaneous injections in the neck two weeks apart. The proteins and bacterial strains doses were diluted in PBS as previously described and administered in a final volume of 100  $\mu$ l for each group of mice. No adverse side effects were observed during the totality of the experimental immunization period. Blood samples were taken just before the first priming injection (preimmune serums) and ten days after the boost immunization (immune serums). The blood aliquots were allowed to clot at room temperature for an hour, centrifuged at 10,000 g for 10 min at 4°C. The sera were harvested and kept at -20°C until subsequent analysis.

**[00255]** *Detection of total IgG, IgG1 and IgG2 by ELISA.* Detection of serum total IgG, IgG1 and IgG2 against each of the antigens previously used for immunization was performed as previously described with some modifications (Ster et al., *Vet. Immunol. Immunopathol.* (2010), 136: 311-318). In addition, detection of IgG against staphylococcal surface protein ClfA was performed to demonstrate the supplementary advantages of using a live strain to enhance and balance the immune response against *S. aureus*. Nunc MaxiSorp™ 96-well plates (Thermo Fisher Scientific Inc., Rochester, NY) were coated with 75  $\mu$ l of each of the test antigen (6.67  $\mu$ g/mL diluted in carbonate/bicarbonate buffer, Sigma Aldrich, Oakville, ON) and incubated overnight at room temperature. The plates were then saturated with PBS containing 5% skim milk powder for 1 h at 37°C. One hundred microliters of four-fold serial dilutions of the sera in PBS containing 3% milk and 0.025% Tween™ 20 were loaded into the plates and incubated for 1 h at 37°C. The plates were washed three times with PBS containing 0.05% Tween™ 20. One hundred microliters of horseradish peroxidase (HRP)-conjugated secondary antibody were then added to the plate. The secondary antibodies used were a goat anti-mouse IgG, IgG2a and IgG1 (Jackson ImmunoResearch Laboratories Inc., West Grove, PA), diluted 1/5000 respectively in PBS containing 3% milk and 0.025% Tween™ 20. After 1 h of incubation at 37°C followed by 3 washes, peroxidase activity was detected with 3,3',5,5'-tetramethylbenzidine (TMB) reagent (KPL Inc., Gaithersburg, MD) according to the manufacturer's recommendations.

**[00256]** *Statistical analysis.* Statistical analysis of the antibody titers and of the correlation was performed using GraphPad Prism™ v6.05.

**EXAMPLE 22: The fusion of antigens and the combination with a live attenuated *S. aureus* strain induces**

**high antibody titers in mice – vaccine #7**

[00257] The antigens and live attenuated *S. aureus* strain  $\Delta 720\Delta hemB$  are produced as described in Example 21. Mice are immunized and IgGs detected as described in Example 21.

[00258] The results in FIG. 16 show that immunization with the SACOL0029-1867 fusion (either when co-administered with other antigens or with a live attenuated strain) induces high and specific antibody responses in mice.

**EXAMPLE 23: The live attenuated *S. aureus* strain significantly improves antibody immune response against some specific antigens – vaccine #7**

[00259] The antigens and live attenuated *S. aureus* strain  $\Delta 720\Delta hemB$  are produced as described in Example 21. Mice are immunized and IgGs detected as described in Example 21.

[00260] The results in FIG. 17 show that immunization with the attenuated live strain  $\Delta 720\Delta hemB$  significantly increases the production of specific IgG antibodies against the SACOL0029 antigen, in comparison to that obtained with IgG antibodies from mice immunized with the protein mix alone.

**EXAMPLE 24: The live attenuated *S. aureus* strain induces significant antibody titers against additional surface proteins of *S. aureus* – vaccine #7**

[00261] The antigens and live attenuated *S. aureus* strain  $\Delta 720\Delta hemB$  are produced as described in Example 21. Mice are immunized and IgGs detected as described in Example 21.

[00262] The results in FIG. 18 show that immunization with the attenuated live strain  $\Delta 720\Delta hemB$  (alone or when co-administered with polypeptide antigens) significantly increases the production of specific antibodies against the staphylococcal surface protein ClfA, compared to that achieved with the protein mix alone composed of SACOL0029, SACOL0442, SACOL0720, and SACOL0029-1867.

**EXAMPLE 25: The live attenuated *S. aureus* strain significantly balances the Th1/Th2 immune response – vaccine #7**

[00263] The antigens and live attenuated *S. aureus* strain  $\Delta 720\Delta hemB$  are produced as described in Example 21.

[00264] Serum IgG2a and IgG1 isotypes against the SACOL0029-1867 fusion protein were detected in serums of vaccinated mice as previously described and the ratio of IgG2a to IgG1 titers of each mouse was determined. IgG2a isotype is associated with the Th1 immune response in mice, whereas IgG1 is a marker for the Th2 response. As described in Example 5, the induction of IgG2 production in cows and the extent of the IgG2 titers in milk significantly correlates with protection of the cows against a challenge with *S. aureus*, as judged by the levels of the corresponding somatic cells (SCC) or bacterial counts (CFU) in milk of the cows (FIG. 4C).

[00265] The results shown in FIGs. 19 and 20 demonstrate that the attenuated live strain  $\Delta 720\Delta hemB$  included in the combination immunization vaccine ( $\Delta 720\Delta hemB$  *S. aureus* administered with SACOL0029, SACOL0442, SACOL0720, and SACOL0029-1867) induces a significantly higher IgG2a/IgG1 antibody ratio against the

SACOL0029-1867 fusion and SACOL0029 proteins than that seen with the protein mix immunization (SACOL0029, SACOL0442, SACOL0720, and SACOL0029-1867), resulting in a significantly more balanced Th1/Th2 response.

**[00266]** Examples 21 to 25 above show that even if a strong antibody response was obtained by the immunization with different antigens (including *e.g.*, SACOL0029-1867 fusion) in a protein mix composition, the immunization of mice with a combination of these antigens with a live attenuated strain significantly improved immune responses against *S. aureus*, by inducing higher antibody titers against some specific antigens (*e.g.*, SACOL0029), by the production of antibodies against other staphylococcal proteins (*e.g.*, ClfA), and by achieving a more balanced IgG2a/IgG1 ratio, a good marker of a stronger Th1 type response, against the antigens co-administered with the live strain.

**EXAMPLE 26: Expression of recombinant proteins in strain *S. aureus*  $\Delta$ hemB $\Delta$ 720 – Vaccines #8, 9, 10 etc.**

**[00267]** Genes SACOL0442, SACOL0720, SACOL0029, and/or SACOL1867 as well as the fusion (*e.g.*, size of 50 AA or more) of the genes (or of fragments thereof) SACOL0029 and SACOL1867 (SACOL0029-SACOL1867), fusions of fragments (*e.g.*, epitopes) of SACOL720 and/or of SACOL0442 (fusion 720-720) (fusion 442-720) or any other fusion of genes or fragments thereof *e.g.*, SACOL0029-SACOL0442, SACOL0029-SACOL0720, SACOL0029-SACOL0720-SACOL0442, SACOL0029-SACOL0720-SACOL1867, SACOL0029-SACOL1867-SACOL0442, SACOL0442-SACOL0029-SACOL0720, SACOL0442-SACOL0029-SACOL0720, SACOL0442-SACOL1867-SACOL0720, SACOL0720-SACOL0442-SACOL1867, SACOL04029-SACOL1867-SACOL0720-SACOL0442, are cloned in plasmid pCN36 (Charpentier *et al.*, 2004) under a constitutive promoter (*PblaZ* from plasmid pCN40) (Charpentier *et al.*, 2004) and expressed in the *S. aureus*  $\Delta$ hemB $\Delta$ 720 strain. Certain protein antigens proposed herein are predicted to be an exotoxin, enterotoxin or superantigen (*e.g.*, SACOL0442) or proteins useful for protection against host defenses (*e.g.*, SACOL0720) and could potentially interfere with the mammalian immune system and antibody production, and/or show some toxicity in the host. Although such interference was not observed with the vaccine composition and formulations described here, it may be useful to modify the protein or polypeptide expressed in the *S. aureus*  $\Delta$ hemB $\Delta$ 720 strain so that the cloned genes do not complement its virulence. For such a purpose, it is possible to use molecular biology techniques to delete or mutate the putative region(s) involved in such protein activity without losing immunogenicity (Chang *et al.*, 2008). This is the approach the applicants used to prepare the antigens of vaccine compositions of the present invention.

**[00268]** Expression of individual recombinant protein products by each of the *S. aureus*  $\Delta$ hemB $\Delta$ 720 strains carrying one of the constructed expression vectors is validated by LC-MS/MS analyses. Briefly, strains grown in BHI with 15  $\mu$ g/ml tetracycline to mid-logarithmic phase were centrifuged and pellets were inactivated with ethanol. Samples were kept at -20°C until cell lysis and trypsin digestion procedures. Samples are incubated with lysostaphin and trypsin at 37°C, followed by cell disruption by mechanical homogenization using glass beads and a beadbeater. Lysates are then centrifuged for 25 min at 13 000 rpm at 4 °C in order to remove cell debris, before

following with protein digestion with trypsin, reduction and alkylation were done by standard procedures before sample injection for protein detection using the MRM method of LC-MS/MS.

[00269] Alternatively, recombinant protein expression is also confirmed by Western blots of bacterial lysates.

**EXAMPLE 27: Mouse immunization with attenuated strains expressing antigens – Vaccines #8, etc.**

[00270] CD-1 female mice are vaccinated by two subcutaneous injections two weeks apart. Each of the *S. aureus*  $\Delta$ hemB $\Delta$ 720 strains carrying or not one of the constructed expression vectors, are diluted in saline and administered in a final volume of 100  $\mu$ l per dose. Group 1 receives a double-mutant strain alone; group 2 receives a double-mutant strain expressing fusion SACOL0029-1867; group 3 receives a double-mutant strain expressing fusion SACOL0029-0442; group 4 receives a double-mutant strain expressing fusion SACOL0029-0720; group 5 receives a mixture of double-mutant strains, one expressing fusion SACOL0029-1867 and the other expressing fusion SACOL0029-0442; group 6 receives a mixture of double-mutant strains, one expressing fusion SACOL0029-1867 and the other expressing fusion SACOL0029-0720; and group 7 receives a mixture of double-mutant strains. Blood samples are taken just before the first injection and twelve days after the second one. The samples are allowed to clot at room temperature for an hour, then centrifuged at 2 000 g for 10 min at 4°C. The supernatants (sera) are harvested and kept at -20°C until subsequent analysis. Mice are euthanized at day 27 and blood is collected by cardiac puncture. The immune sera are recovered, aliquoted and stored as for the pre-immune sera.

[00271] The immune response to vaccination is evaluated by enzyme-linked immunosorbent assay (ELISA) for the presence of serum polyclonal IgG antibodies directed towards *S. aureus* whole cells (Wood strain) or specific recombinant proteins. Anti-mouse IgG-HRP (HRP: horseradish peroxidase) is used as a secondary antibody to detect the colorimetric production of 3,3',5,5'-tetramethylbenzidine (TMB) substrate oxidation by peroxidase activity using a spectrophotometer.

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**CLAIMS**

1. A fusion construct of formula (I):

X-A-linker-B-Z (I)

wherein :

(1) A and B are identical or different and are independently:

- (a) a polypeptide comprising a SACOL0029 polypeptide as set forth in any one of the sequences depicted in FIG. 24 (SEQ ID NOs: 5 and 121 to 131), a SACOL0264 polypeptide (SEQ ID NO: 185), a SACOL0442 polypeptide as set forth in any one of the sequences depicted in FIG. 22D (SEQ ID NOs: 29 and 82 to 92), a SACOL0718 polypeptide (SEQ ID NO: 186), a SACOL0720 polypeptide as set forth in any one of the sequences depicted in FIGs. 23I-K (SEQ ID NOs: 11 and 109 to 120), a SACOL1353 polypeptide (SEQ ID NO : 187), a SACOL1416 polypeptide (SEQ ID NO : 188), a SACOL1611 polypeptide (SEQ ID NO : 189), a SACOL1867 polypeptide as set forth in any one of the sequences depicted in FIG. 25D (SEQ ID NOs: 152 to 164), a SACOL1912 polypeptide (SEQ ID NO : 43), a SACOL1944 polypeptide (SEQ ID NO : 190), a SACOL2144 polypeptide (SEQ ID NO : 191), a SACOL2365 polypeptide (SEQ ID NO : 192), a SACOL2385 polypeptide (SEQ ID NO : 50) or a SACOL2599 polypeptide (SEQ ID NO : 193), based on the gene nomenclature from the *Staphylococcus aureus* COL (SACOL) genome set forth in NCBI Reference Sequence NC\_002951.2;
- (b) a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a);
- (c) a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a) or (b);
- (d) a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a) to (c); or
- (e) a polypeptide comprising an immunogenic variant comprising at least 13 consecutive amino acids of any one of (a) to (c);

(2) the linker is an amino acid sequence of at least one amino acid or is absent;

(3) X is absent or is an amino acid sequence of at least one amino acid; and

(4) Z is absent or is an amino acid sequence of at least one amino acid.

2. The construct of claim 1, wherein (1) (a) is a polypeptide comprising a SACOL0029 polypeptide as set forth in any one of the sequences depicted in FIG. 24 (SEQ ID NOs: 5 and 121 to 131), a SACOL0442 polypeptide as set forth in any one of the sequences depicted in FIG. 22D (SEQ ID NOs: 29 and 82 to 92), a SACOL0720 polypeptide as set forth in any one of the sequences depicted in FIGs. 23I-K (SEQ ID NOs: 11 and 109 to

120), or a SACOL1867 polypeptide as set forth in any one of the sequences depicted in FIG. 25D (SEQ ID NOs: 152 to 164).

3. The construct of claim 2, wherein at least one of A and B is (a) a polypeptide comprising a SACOL0029 polypeptide as set forth in any one of the sequences depicted in FIG. 24 (SEQ ID NOs: 5 and 121 to 131); (b) a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a); (c) a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a) or (b); (d) a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a) to (c); or (e) a polypeptide comprising an immunogenic variant comprising at least 13 consecutive amino acids of any one of (a) to (c); and the other one of A and B is (a') a polypeptide comprising a SACOL1867 polypeptide as set forth in any one of the sequences depicted in FIG. 25D (SEQ ID NOs: 152 to 164); (b') a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a'); (c') a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a') or (b'); (d') a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a') to (c'); or (e') a polypeptide comprising an immunogenic variant comprising at least 12 consecutive amino acids of any one of (a') to (c').
4. The construct of claim 2, wherein at least one of A and B is (a) a polypeptide comprising a SACOL0442 polypeptide as set forth in any one of the sequences depicted in FIG. 22D (SEQ ID NOs: 29 and 82 to 92); (b) a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a); (c) a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a) or (b); (d) a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a) to (c); or (e) a polypeptide comprising an immunogenic variant comprising at least 13 consecutive amino acids of any one of (a) to (d); and the other one of A and B is (a') a polypeptide comprising a SACOL0720 polypeptide as set forth in any one of the sequences depicted in FIGs. 23I-K (SEQ ID NOs: 11 and 109 to 120); (b') a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a'); (c') a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a') or (b'); (d') a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a') to (c'); or (e') a polypeptide comprising an immunogenic variant comprising at least 12 consecutive amino acids of any one of (a') to (d').
5. The construct of claim 2, wherein A and B are identical or different and are (a) a polypeptide comprising a SACOL0720 polypeptide as set forth in any one of the sequences depicted in FIGs. 23I-K (SEQ ID NOs: 11 and 109 to 120); (b) a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a); (c) a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a) or (b); (d) a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a) to (c); or (e) a polypeptide comprising an immunogenic variant comprising at least 13 consecutive amino acids of any one of (a) to (d).
6. The construct of any one of claims 1-2 and 4, wherein said immunogenic fragment (d) comprises one or more of the following amino acid sequences: KDTINGKSNKSRNW (SEQ ID NO: 34); and KDGGKYTLESHKELQ

(SEQ ID NO: 1).

7. The construct of claim 6, wherein said immunogenic fragment (d) comprises one or more of the following amino acid sequences :  
 STQNSSSVQDKQLQKVEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIHLEGTYTV  
 AGRVYTPKRNITLNKEVVTLKELDHIIIRFAHISYGLYMGEHLPKGNIVINTKDGGKYTLESHKELQKDRENVKINT  
 ADIKNVTFKLKSVNDIEQV (SEQ ID NO: 30);  
 DKQLQKVEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIHLEGTYTVAGRVYTPKR  
 NITLNKEVVTLKELDHIIIRFAHISYGLYMGEHLPKGNIVINTK (SEQ ID NO :32); and  
 DKQLQKVEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIHLEGTYTVAGRVYTPKR  
 NITLNKEVVTLKELDHIIIRFAHISYGLYMGEHLPKGNIVINTKDGGKYTLESHKELQKDRENVKINTADIKNVTFKL  
 KSVNDIEQV (SEQ ID NO : 33).
8. The construct of any one of claims 1-2 and 4-5, wherein said immunogenic fragment (d) comprises one or more of the following amino acid sequences: QFGFDLKHKKDALA (SEQ ID NO: 21); TIKDQQKANQLAS (SEQ ID NO: 22); KDINKIYFMTDVL (SEQ ID NO: 23); and DVDLGGPTFVLND (SEQ ID NO: 24).
9. The construct of claim 8, wherein said immunogenic fragment (d) comprises one or more of the following amino acid sequences:  
 RASLSSEIKYTAPHDVTIKDQQKANQLASELNNQKIPHFYNYKEVIHTKLYKDNLFDVKAKEPYNVTITSDKYIP  
 NTDLKRGGADLFVAEGSIKDLVKHKKHGKAIIGTKKHHVNIKLKRDINKIYFMTDVLGGPTFVLNDKDYQEIRK  
 YTKAHIVSQFGFDLKHKKDALALEKAKNKVDKSIETRSEAISSISLTG (SEQ ID NO : 12);  
 ASLSSEIKYTAPHDVTIKDQQKANQLASELNNQKIPHFYNYKEVIHTKLYKDNLFDVKAKEPYNVTITSDKYIPNT  
 DLKRGGADLFVAEGSIKDLVKHKKHGKAIIGTKKHHVNIKLKRDINKIYFMTDVLGGPTFVLNDKDYQEIRKYT  
 KAHIVSQFGFDLKHKKDALALEKAKNKVDKSIETRSEAISSISLTG (SEQ ID NO: 13);  
 ASLSSEIKYTAPHDVTIKDQQKANQLASELNNQKIPHFYNYKEVIHTKLYKDNLFDVKAKEPYNVTITSDKYIPNT  
 DLKRGGADLFVAEGSIKDLVKHKKHGKAIIGTKKHHVNIKLKRDINKIYFMTDVLGGPTFVLNDKDYQE (SEQ  
 ID NO: 14); KDINKIYFMTDVLGGPTFVLNDKDYQEIRKYTKAHIVSQFGFDLKHKKDALA (SEQ ID NO : 15);  
 KDINKIYFMTDVLGGPTFVLNDKDY (SEQ ID NO : 17); KDINKIYFMTDVLGGPTFVLNDKD (SEQ ID NO :  
 16); KDINKIYFMTDVLGGPTFVLND (SEQ ID NO : 19); KHIVSQFGFDLKHKKDALA (SEQ ID NO: 20) and  
 SQFGFDLKHKKDALA (SEQ ID NO: 18).
10. The construct of any one of claims 1-3, wherein said immunogenic fragment (c') comprises one or more of the following amino acid sequences: PYNGVVSFKDATGF (SEQ ID NO: 165); AHPNGDKGNGGIYK (SEQ ID NO: 167); SISDYPGDEDISVM (SEQ ID NO: 169); RGPKGFNFNENVQA (SEQ ID NO: 172); QFESTGTIKRIKDN (SEQ ID NO: 175); and GNSGSPVLNSNNEV (SEQ ID NO: 178).
11. The construct of claim 10, wherein said immunogenic fragment (d) comprises the following amino acid sequence:  
 TQVKDTNIFPYNGVVSFKDATGFVIGKNTIITNKHVSKDYKVGDRITAHPNGDKGNGGIYKIKSISDYPGDEDIS  
 VMNIEEQAVERGPKGFNFNENVQAFNFAKDAKVDDKIKVIGYPLPAQNSFKQFESTGTIKRIKDNILNFDAYIEP

GNSGSPVLNSNNEVIGVYGGIGKIGSEYNGAVYFTPQIKDFIQKHIEQ (SEQ ID NO: 39).

12. The construct of any one of claims 1 to 11, wherein the linker comprises at least four identical or different amino acids selected from the group consisting of glycine, serine, alanine, aspartate, glutamate and lysine.
13. The construct of any one of claims 1 to 12, wherein the linker comprises (GGGGS)<sub>n</sub> (SEQ ID NO: 67), (ERKYK)<sub>n</sub> (SEQ ID NO: 61); or (EAAAK)<sub>n</sub> (SEQ ID NO: 63), wherein n=1 to 5.
14. The construct of any one of claims 1 to 13, wherein said X comprises a polyhistidine of 6 to 10 amino acids.
15. The construct of any one of claims 1 to 13, wherein said X is absent.
16. The construct of any one of claims 1 to 15, wherein said Z is absent.
17. An isolated nucleic acid molecule encoding the construct defined in any one of claims 1 to 16.
18. A vector comprising the isolated nucleic acid defined in claim 17.
19. A host cell comprising the vector defined in claim 18.
20. The cell of claim 19, which is a live attenuated form of *Staphylococcus aureus*.
21. The cell of claim 20, wherein the live attenuated form of *Staphylococcus aureus* has a stabilized small colony variant (SCV) phenotype.
22. The cell of claim 21, wherein the live attenuated form of *Staphylococcus aureus* having a stabilized SCV phenotype is a  $\Delta hemB\Delta 720$  *S. aureus*.
23. A composition comprising:
  - (A) at least one of the constructs defined in any one of claims 1 to 16; at least one of the nucleic acid molecules defined in claim 17; at least one of the vectors defined in claims 18; or at least one of the cells defined in any one of claims 19 to 22; and
  - (B) (i) a polypeptide defined in any one of claims 1 to 11;
    - (ii) a live attenuated *Staphylococcus aureus*;
    - (iii) a pharmaceutically acceptable excipient;
    - (iv) an adjuvant; or
    - (v) a combination of at least two of (i) to (iv).
24. The composition of claim 23, wherein the live attenuated form of *Staphylococcus aureus* expresses:
  - (a) a polypeptide comprising a SACOL0029 polypeptide as set forth in any one of the sequences depicted in FIG. 24 (SEQ ID NOs: 5 and 121 to 131), a SACOL0264 polypeptide (SEQ ID NO: 185), a SACOL0442 polypeptide as set forth in any one of the sequences depicted in FIG. 22D (SEQ ID NOs: 29 and 82 to 92), a SACOL0718 polypeptide (SEQ ID NO: 186), a SACOL0720 polypeptide as set forth in any one of the sequences depicted in FIGs. 23I-J (SEQ ID NOs: 11 and 109 to 120), a

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SACOL1353 polypeptide (SEQ ID NO : 187), a SACOL1416 polypeptide (SEQ ID NO : 188), SACOL1611 (SEQ ID NO : 189), a SACOL1867 polypeptide as set forth in any one of the sequences depicted in FIG. 25D (SEQ ID NOs: 152 to 164), a SACOL1912 polypeptide (SEQ ID NO : 43), SACOL1944 (SEQ ID NO : 190), a SACOL2144 polypeptide (SEQ ID NO : 191), a SACOL2365 polypeptide (SEQ ID NO : 192), a SACOL2385 polypeptide (SEQ ID NO : 50) or a SACOL2599 polypeptide (SEQ ID NO : 193) based on the gene nomenclature from the *Staphylococcus aureus* COL (SACOL) genome set forth in NCBI Reference Sequence NC\_002951.2;

- (b) a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a);
  - (c) a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a) or (b);
  - (d) a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a) to (c); or
  - (e) a polypeptide comprising an immunogenic variant comprising at least 13 consecutive amino acids of any one of (a) to (c).
25. The composition of claim 23 or 24, wherein the live attenuated form of *Staphylococcus aureus* has a stabilized small colony variant (SCV) phenotype.
26. The composition of any one of claims 23 to 25, wherein the adjuvant comprises alum, an oil, saponin, cyclic-diguanosine-5'-monophosphate (c-di-GMP), polyphosphasine, indolicidin, pathogen-associated molecular patterns (PAMPS), liposome or a combination of at least two thereof.
27. A method for preventing and/or treating a Staphylococcal intramammary infection (IMI) in a mammal, said method comprising administering to said mammal an effective amount of the construct defined in any one of claims 1 to 16; of the nucleic acid molecule defined in claim 17; of the vector defined in claim 18; of the cell defined in any one of claims 19 to 22; or of the composition defined in any one of claims 23 to 26.
28. The method of claim 27, wherein said Staphylococcal IMI is caused by one or more *Staphylococcus aureus* strains.
29. The method of claim 27 or 28, wherein said mammal is a cow.
30. A use of an effective amount of (i) the construct defined in any one of claims 1 to 16; (ii) the nucleic acid molecule defined in claim 17; (iii) the vector defined in claim 18; of the cell defined in any one of claims 19 to 22; (iv) the composition defined in any one of claims 23 to 26; or (v) a combination of at least two of (i) to (iv), for preventing and/or treating a Staphylococcal intramammary infection (IMI) in a mammal.
31. The use of claim 30, wherein said Staphylococcal IMI is caused by one or more *Staphylococcus aureus* strains.
32. The use of claim 30 or 31, wherein said mammal is a cow.
33. The construct defined in any one of claims 1 to 16; the nucleic acid molecule defined in claim 17; the vector

defined in claim 18; the cell defined in any one of claims 19 to 22; the composition defined in any one of claims 23 to 26 or a combination of at least two thereof, for use in the prevention and/or treatment of a Staphylococcal intramammary infection (IMI) in a mammal.

34. The construct, nucleic acid molecule, vector, cell, composition or combination of claim 33, wherein said Staphylococcal IMI is caused by one or more *Staphylococcus aureus* strains.
35. The construct, nucleic acid molecule, vector, cell or composition of claim 33 or 34, wherein said mammal is a cow.
36. A kit for preventing and/or treating a Staphylococcal intramammary infection (IMI) in a mammal comprising:
- (A) (i) at least one of the constructs defined in any one of claims 1 to 16; (ii) at least one of the nucleic acid molecules defined in claim 17; (iii) at least one of the vectors defined in claim 18; (iv) at least one of the cells defined in any one of claims 19 to 22; or (v) a combination of at least two of (i) to (iv), and
  - (B) (i) the polypeptide defined in any one of claims 1 to 11;
    - (ii) a live attenuated *Staphylococcus aureus*;
    - (iii) a pharmaceutically acceptable excipient;
    - (iv) an adjuvant;
    - (v) instructions for using the kit for preventing and/or treating a Staphylococcal intramammary infection (IMI) in a mammal; or
    - (vi) a combination of at least two of (i) to (v).

FIG. 1A

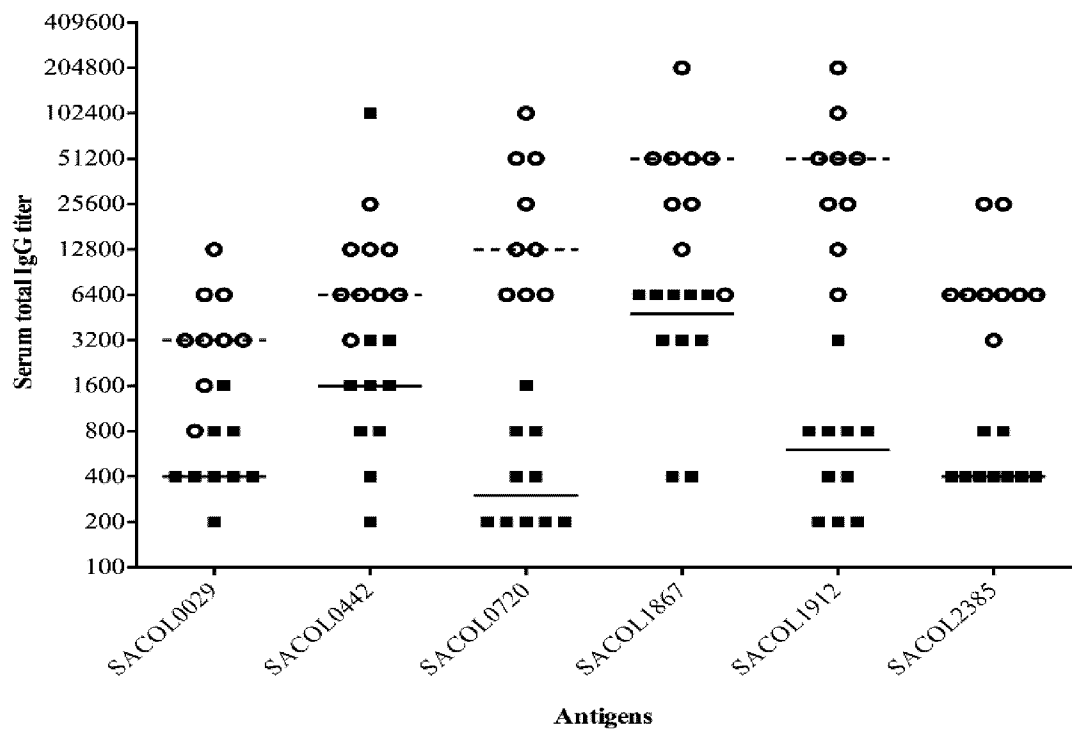


FIG. 1B

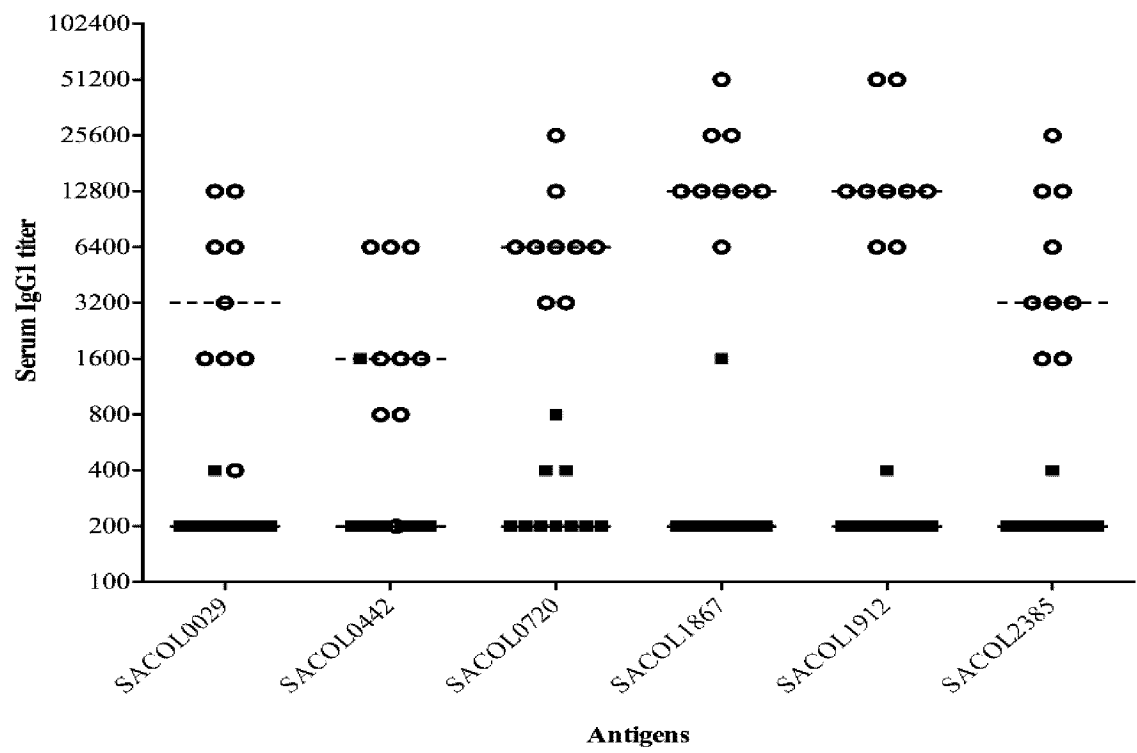


FIG. 1C

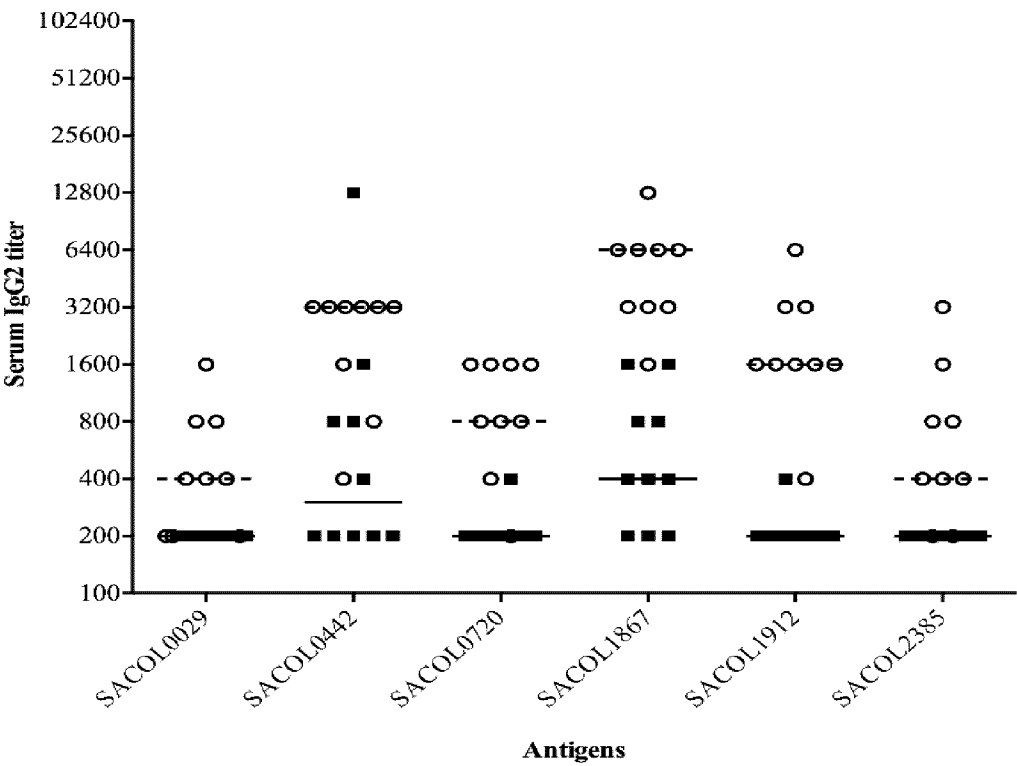
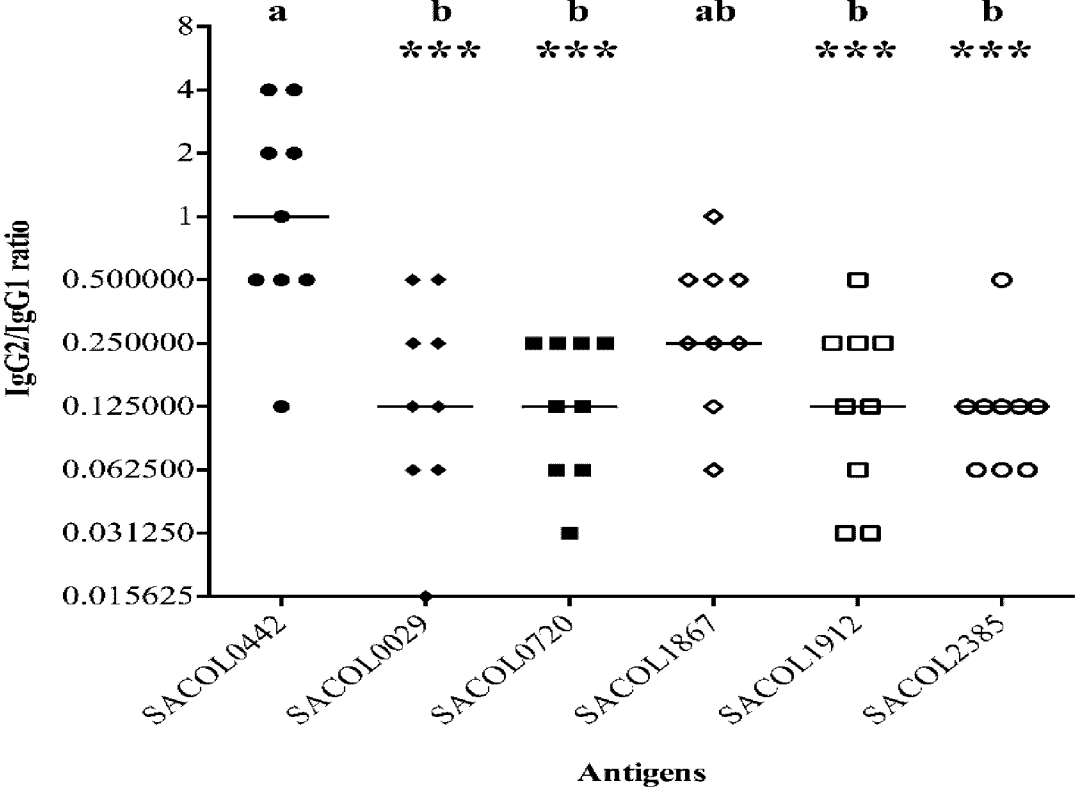
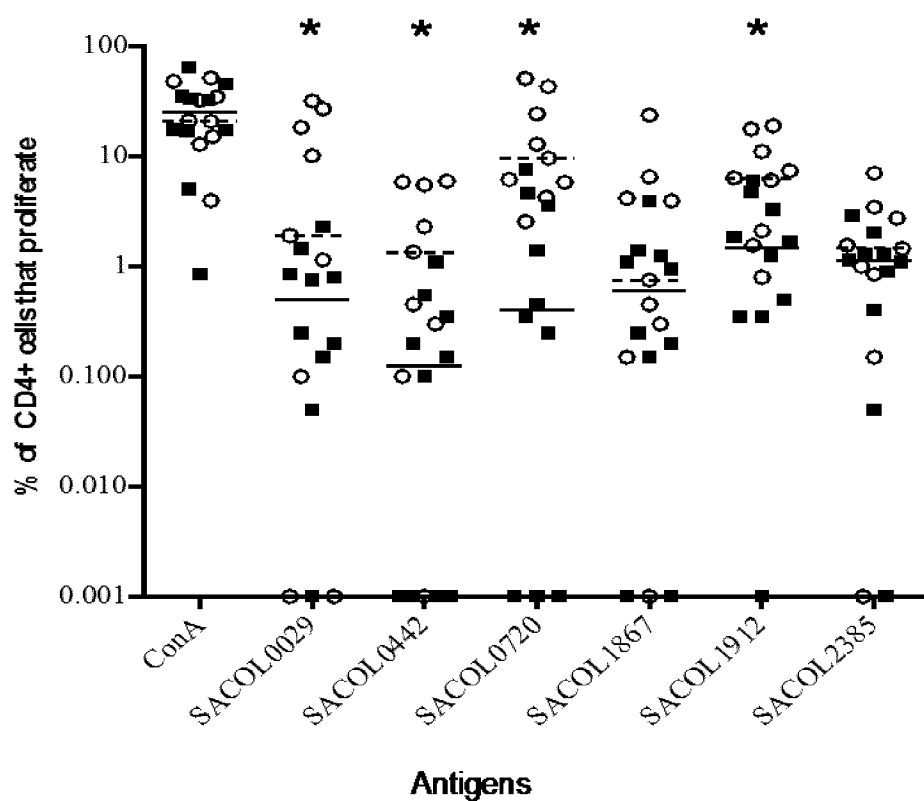


FIG. 1D





Antigens

FIG. 2

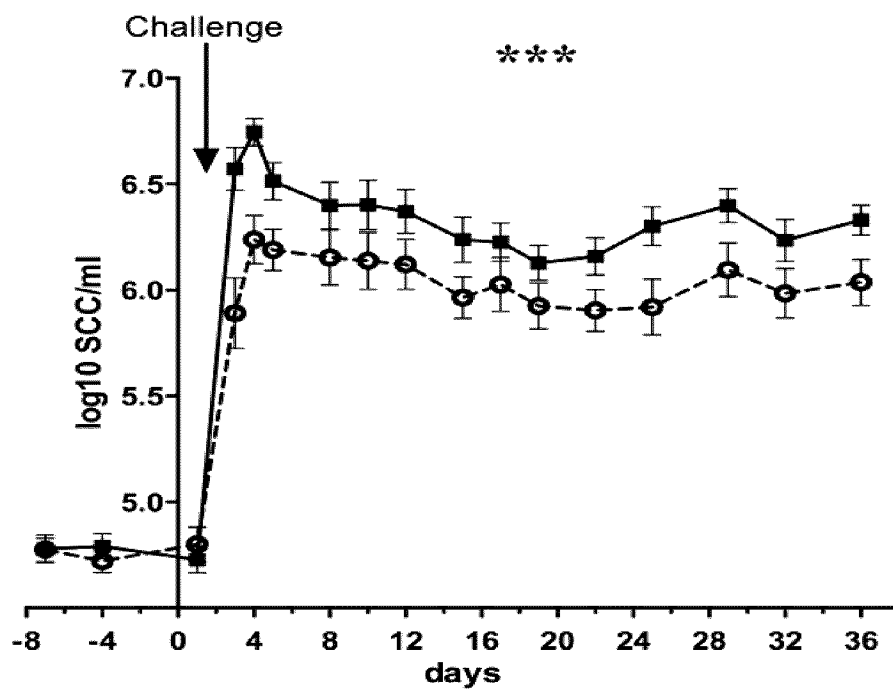


FIG. 3

FIG. 4A

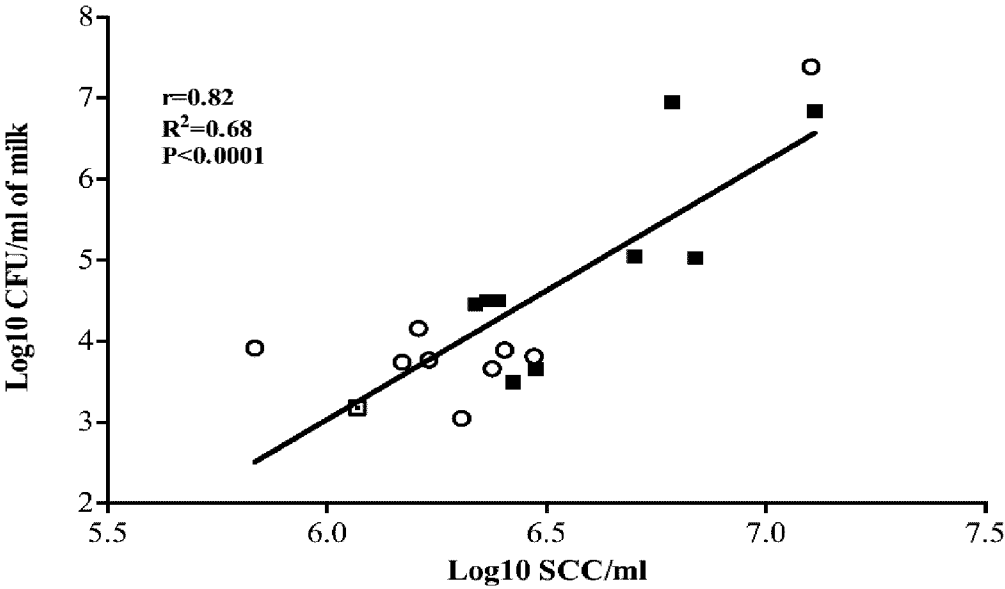


FIG. 4B

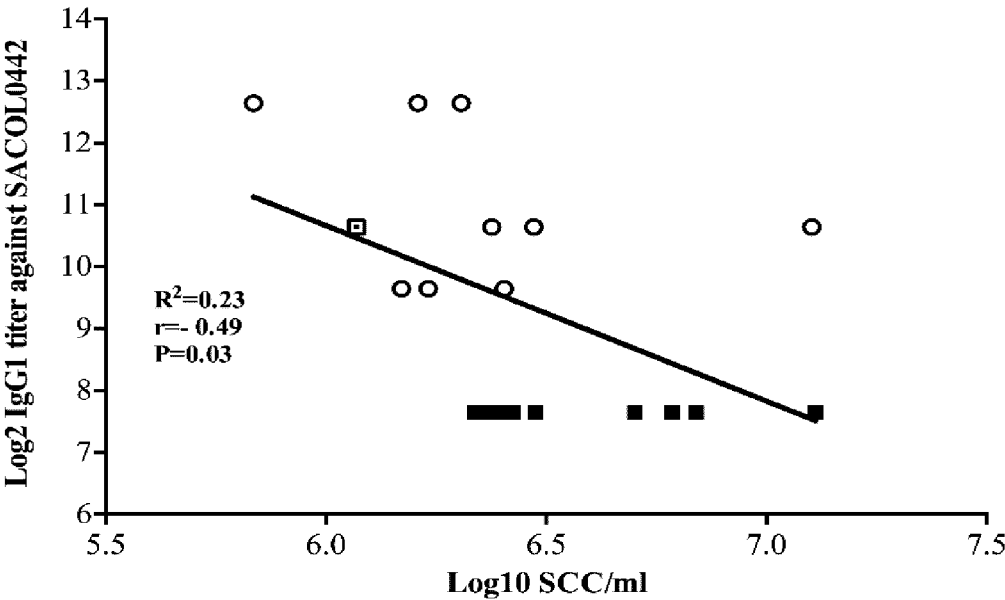


FIG. 4C

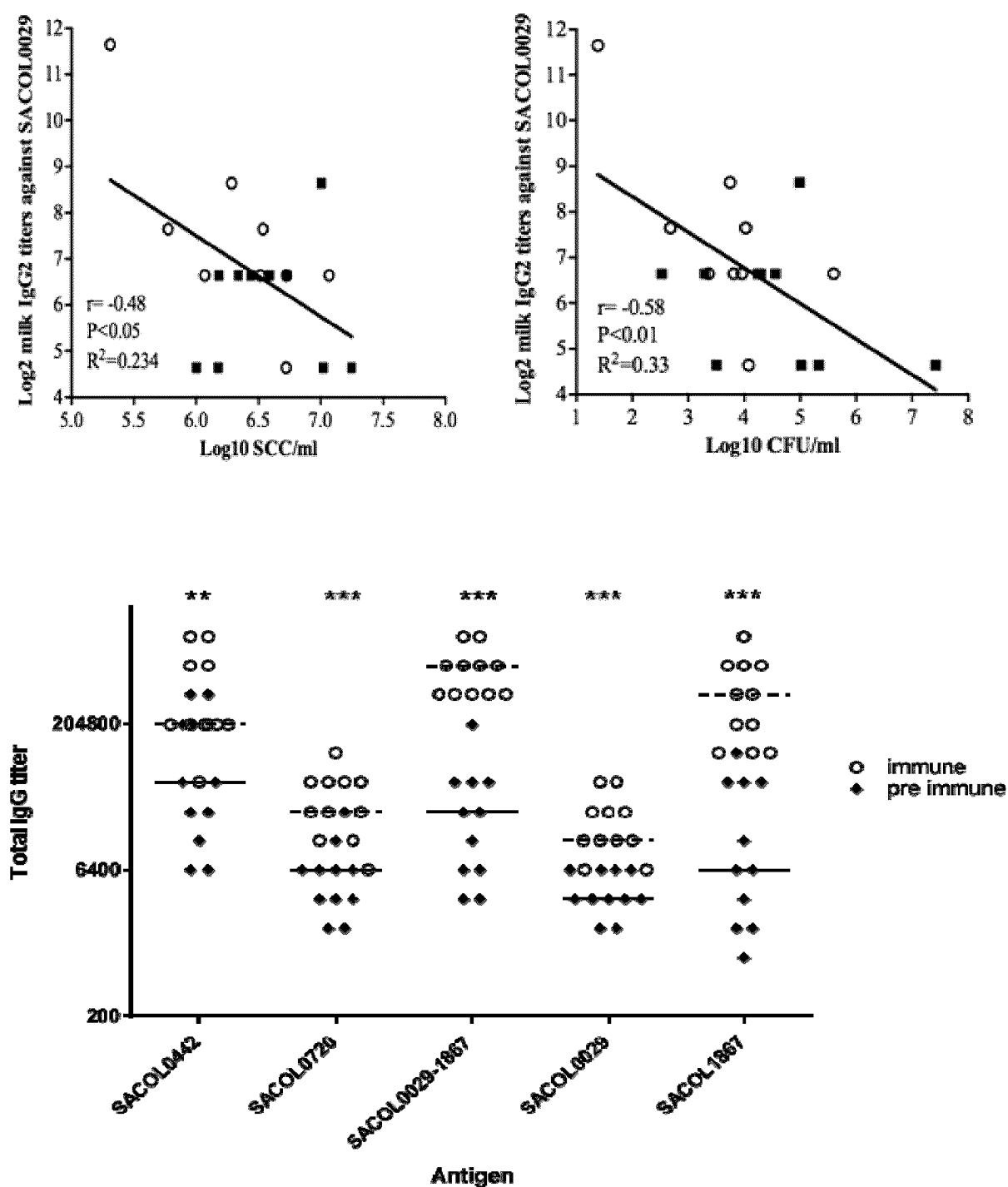


FIG. 5

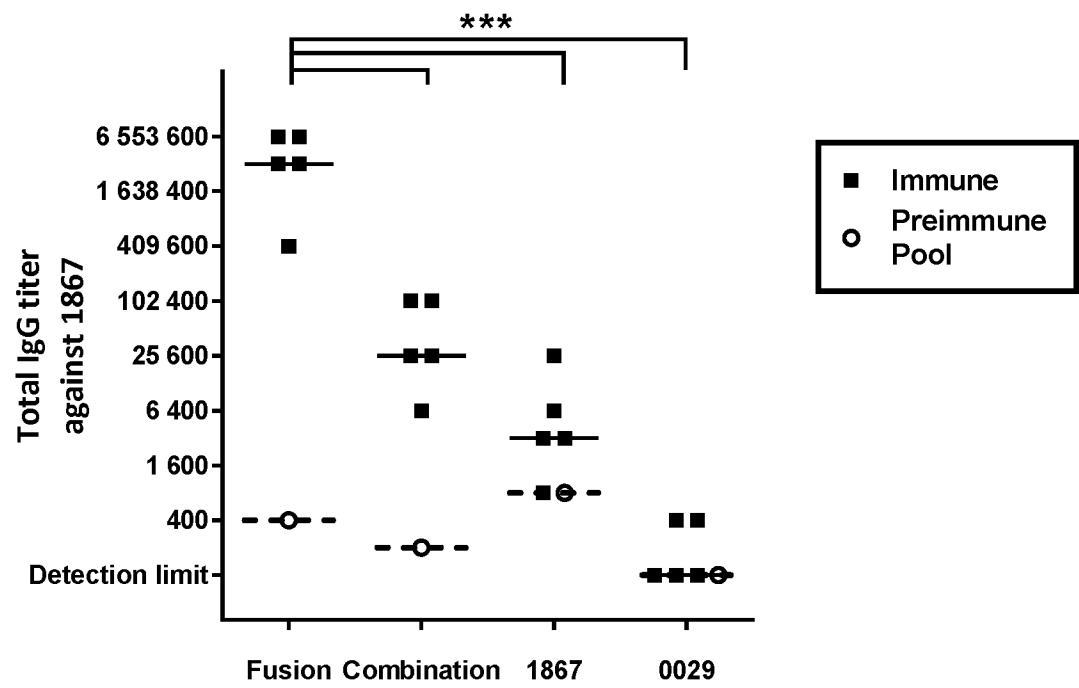


FIG. 6

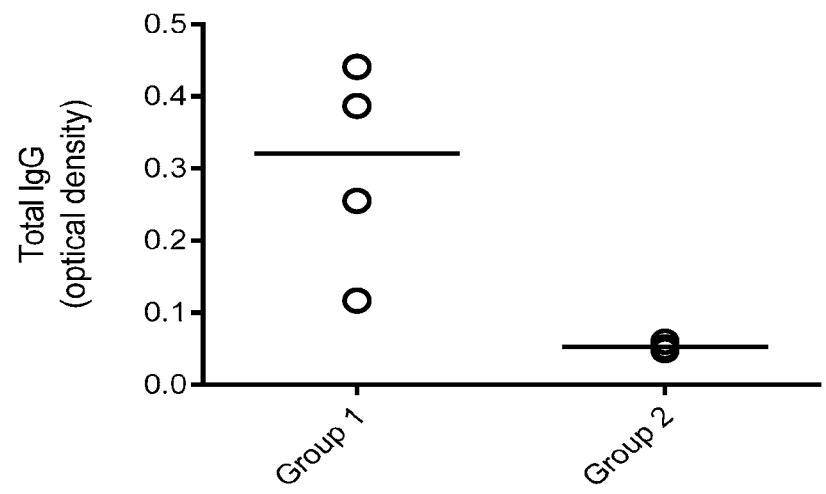


FIG. 7

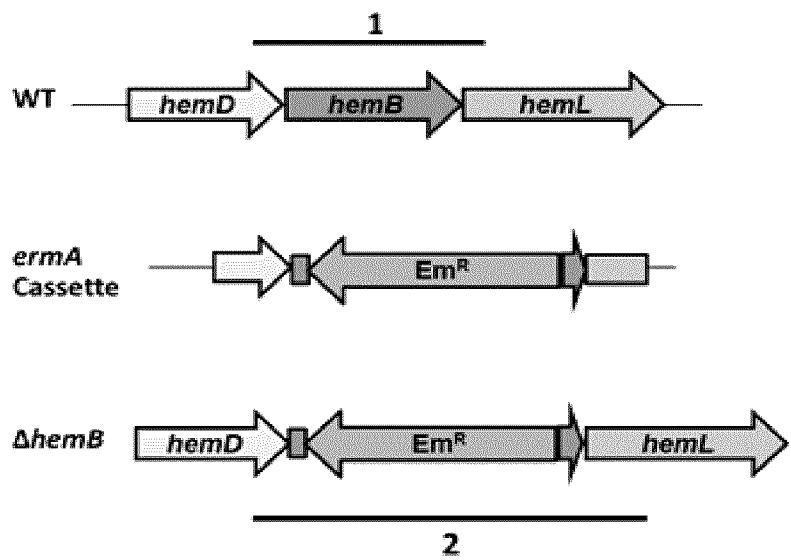


FIG. 8A

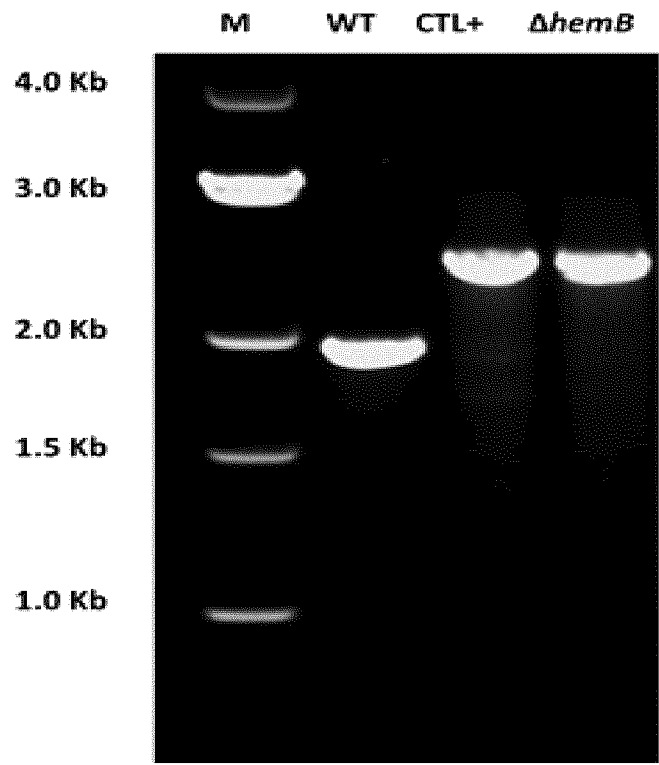


FIG. 8B

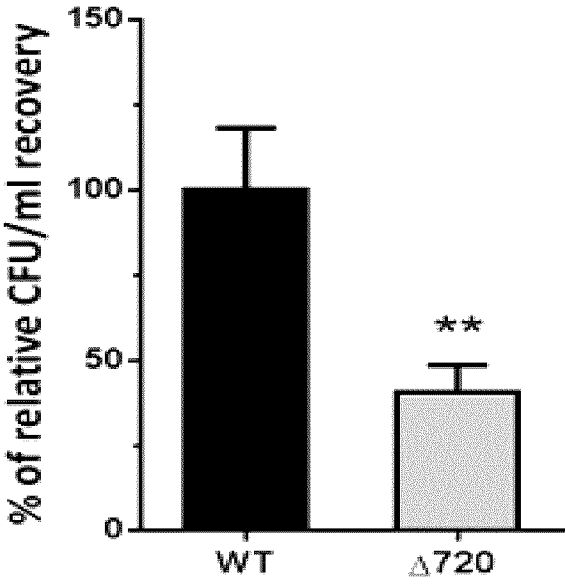


FIG. 9A

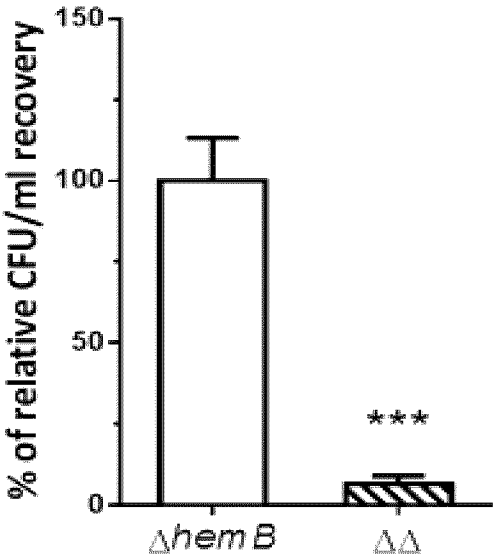


FIG. 9B

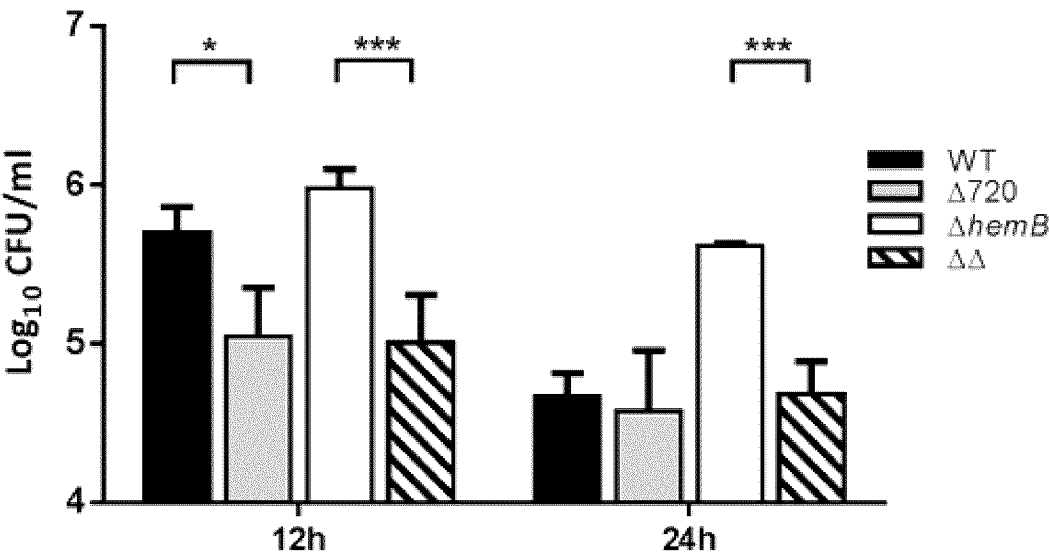


FIG. 9C

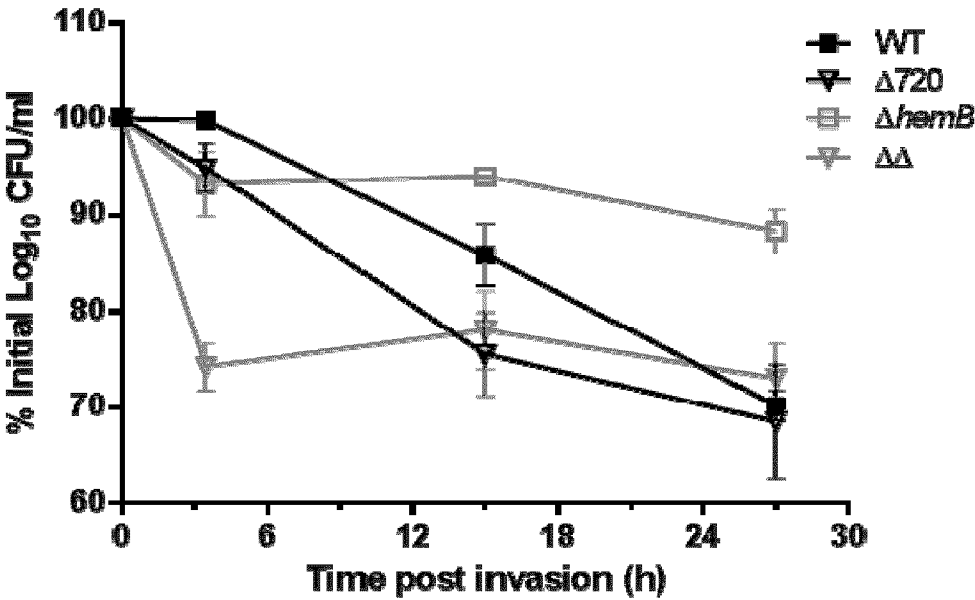


FIG. 10

FIG. 11A

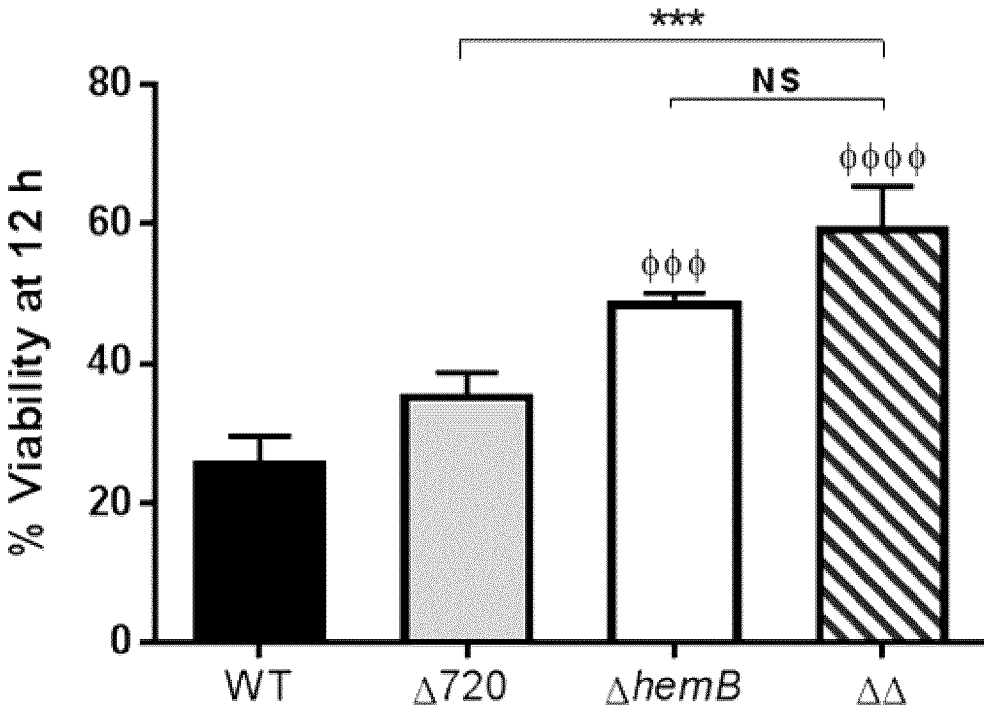
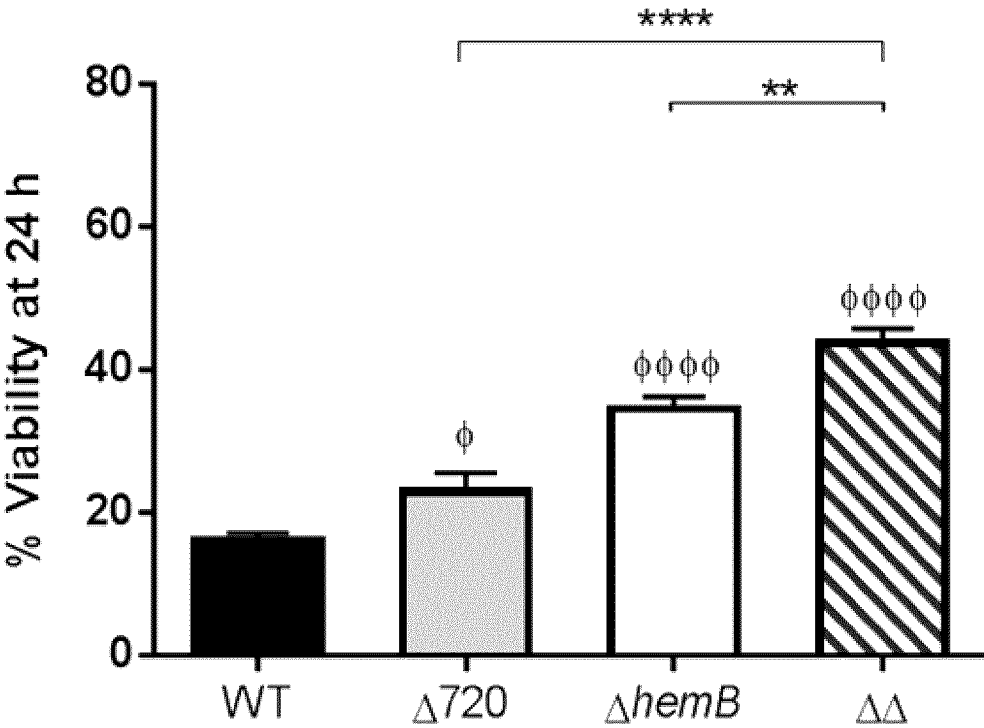


FIG. 11B



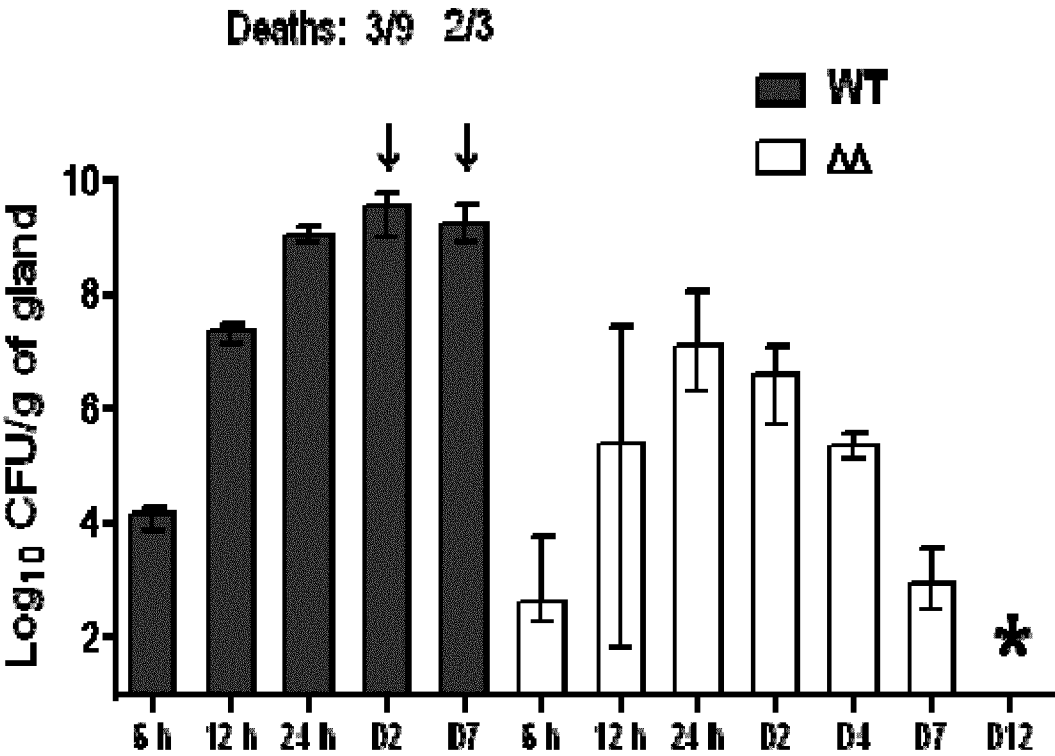


FIG. 12

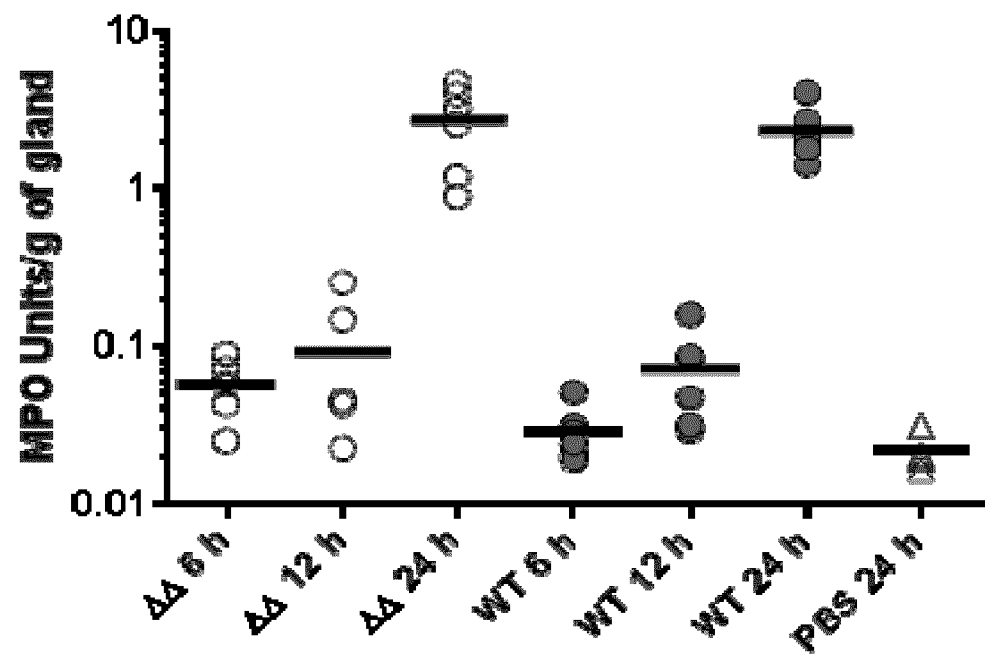


FIG. 13

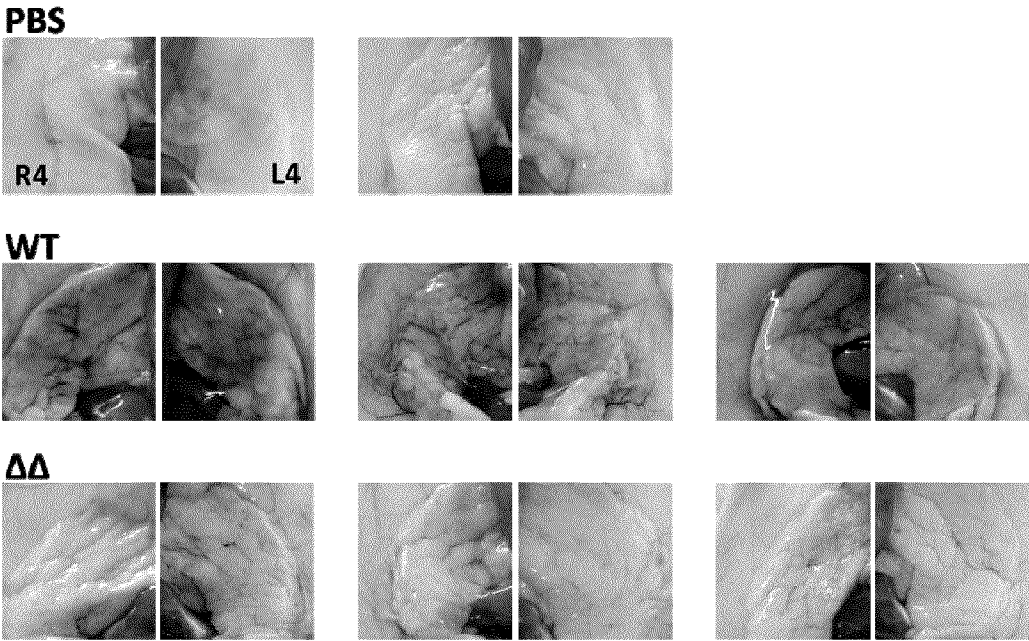


FIG. 14

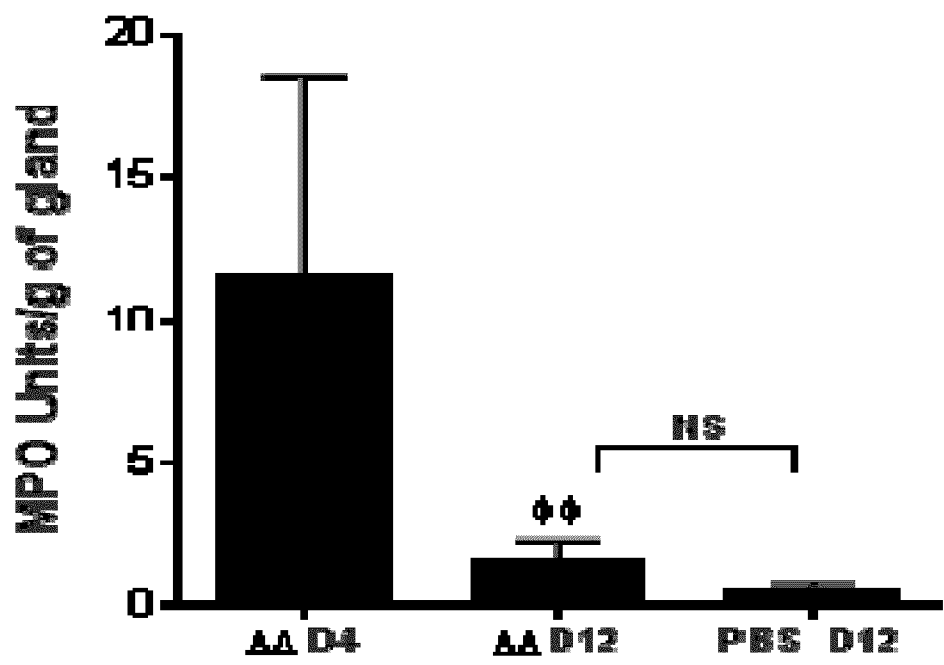


FIG. 15A

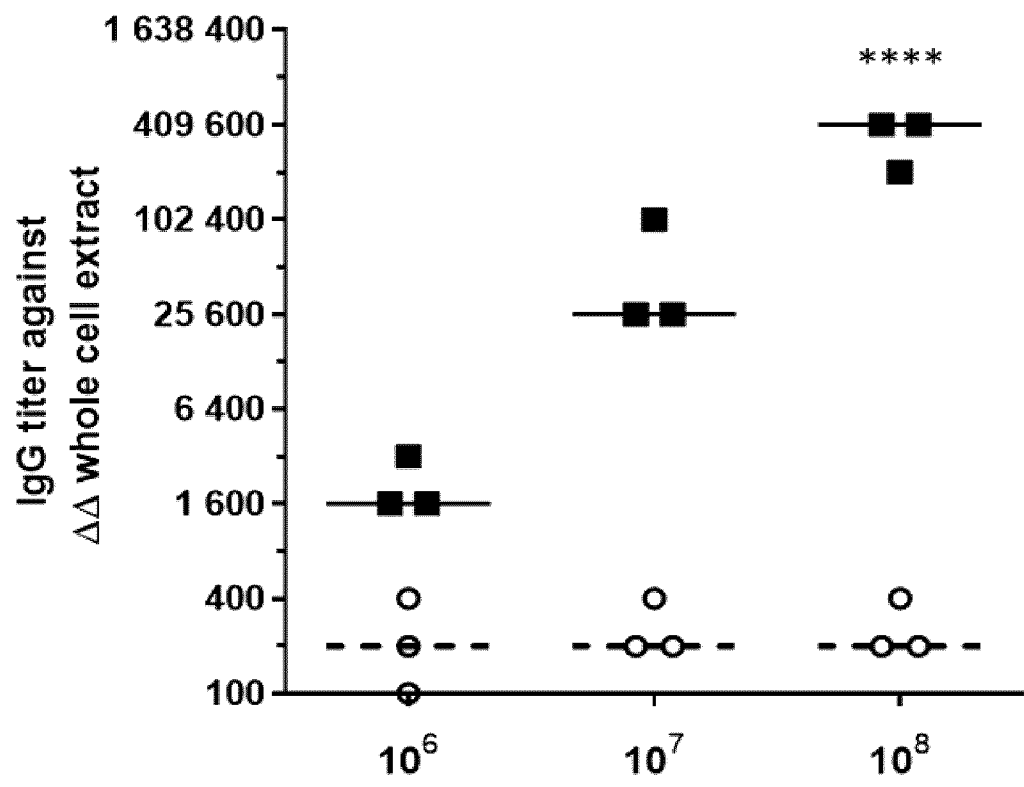


FIG. 15B

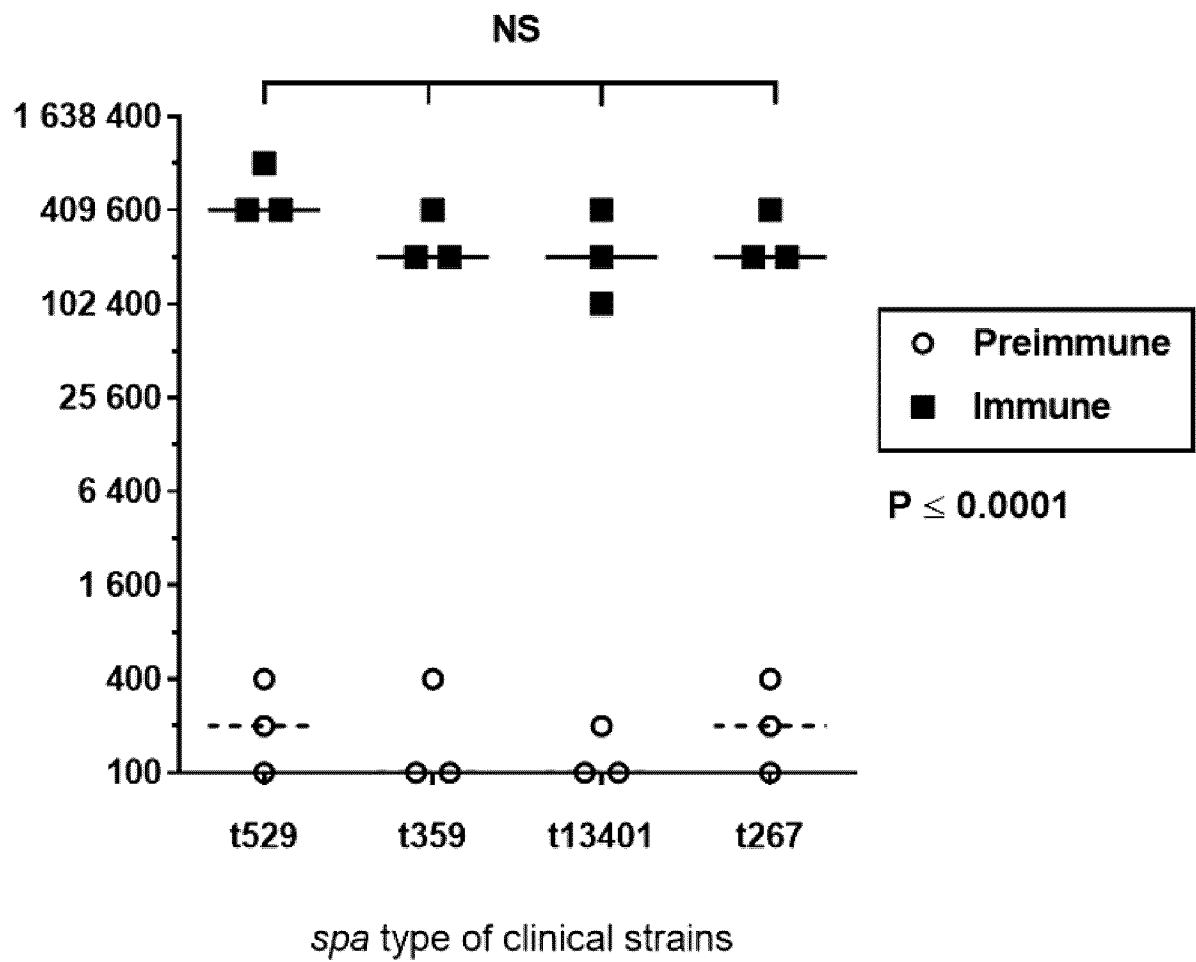


**FIG. 15C**

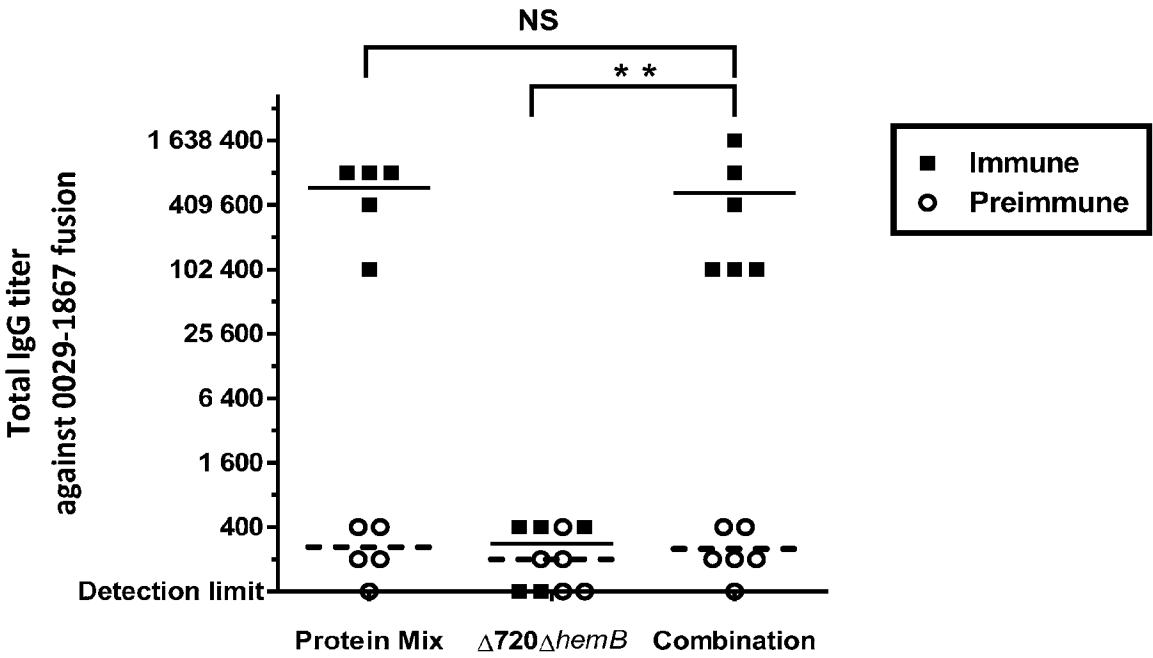


FIG. 16

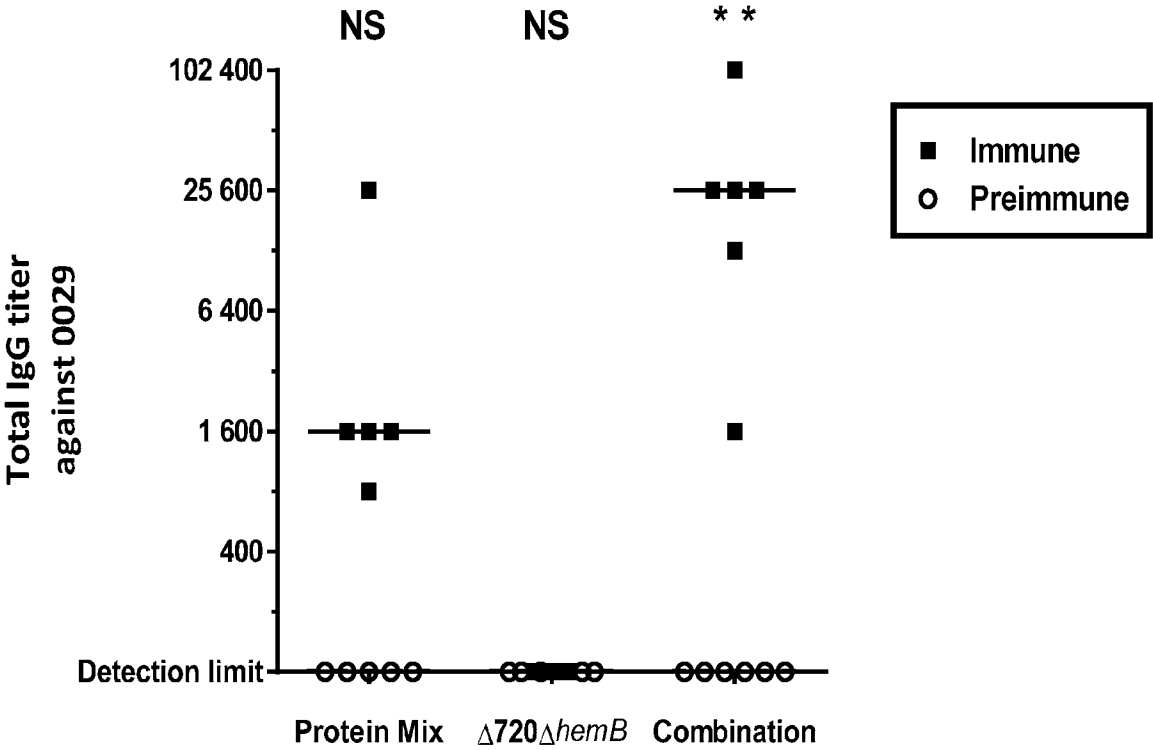


FIG. 17

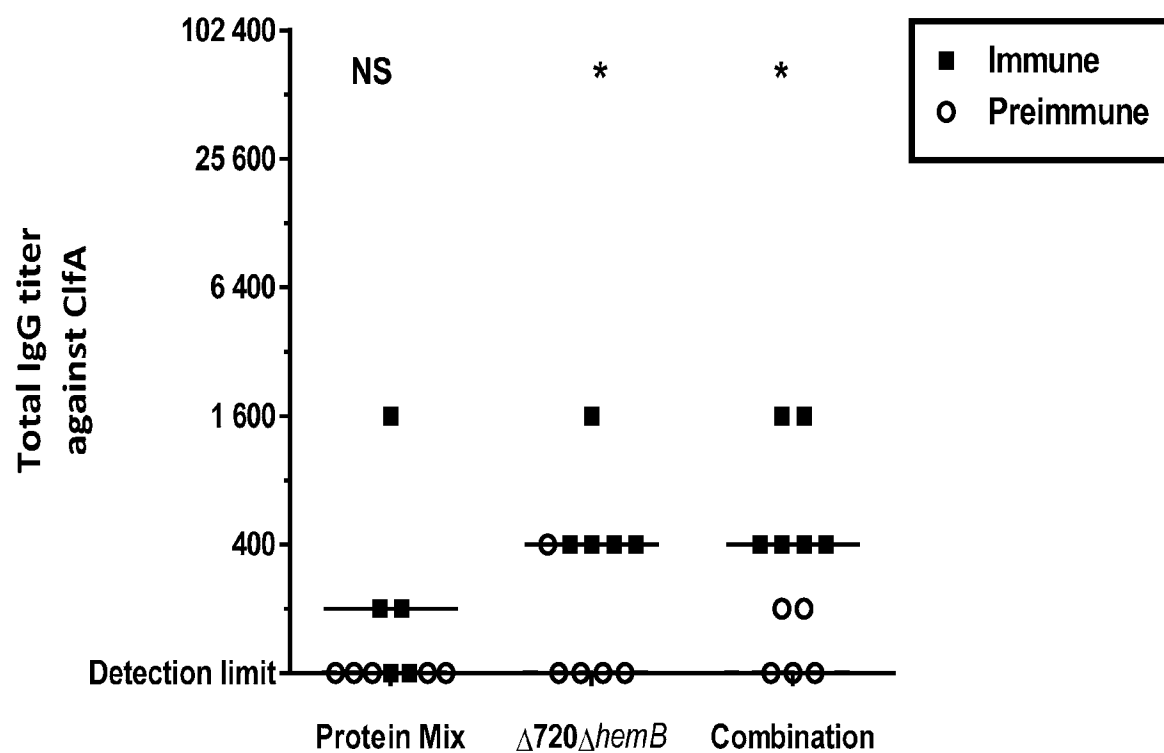


FIG. 18

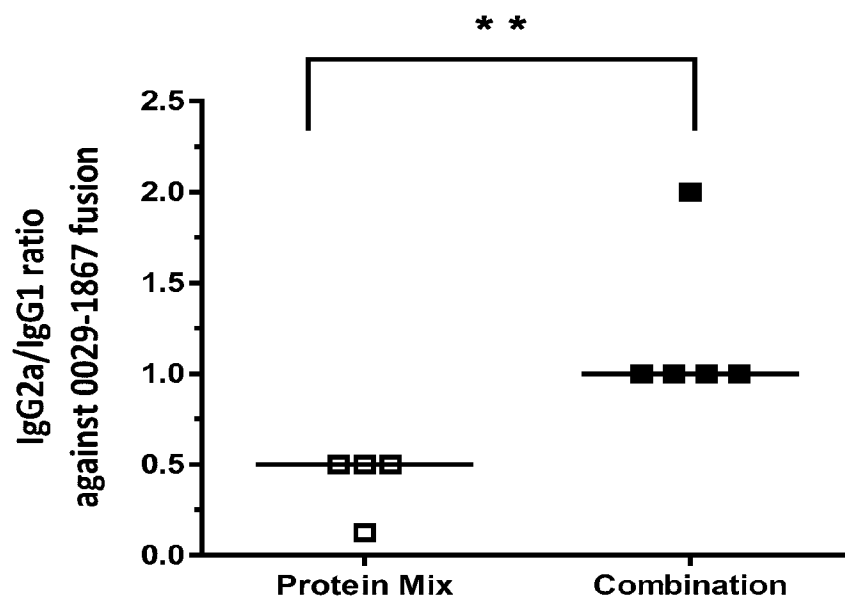


FIG. 19

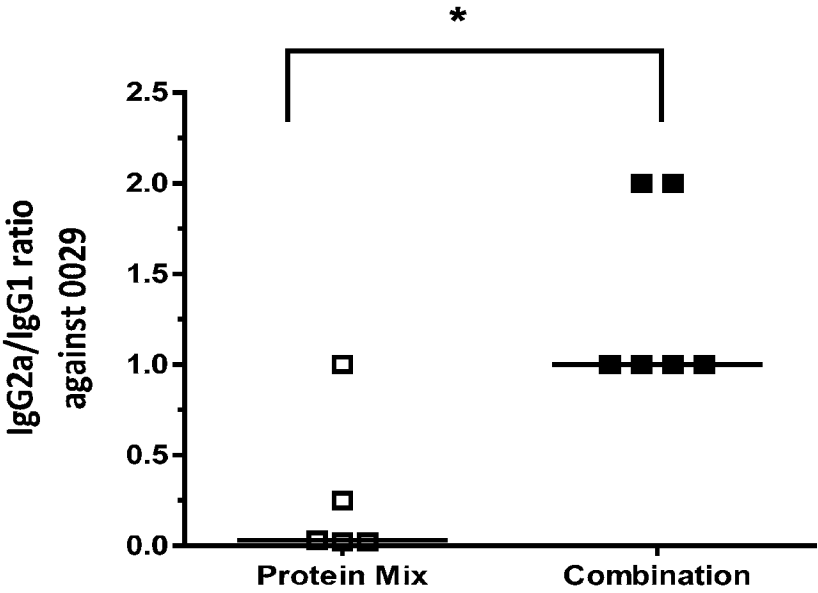


FIG. 20

I. SACOL0029FULL LENGTHPOLYNUCLEOTIDE

&gt;SEQ ID NO:4 (GENBANK GENE ID 3236748)

ATGCTAGAATCTAGAGAGCAATTATCAGTCGAAGAATACGAAACATTCTTTAACAGATTTGATAATCAAGAATTTGATTT  
 CGAACGTGAATTGACACAAGATCCATATTCAAAAGTATACTTATACAGTATAGAAGACCATATCAGAACATATAAGATAG  
 AGAAATAA

POLYPEPTIDE

&gt;SEQ ID NO: 5 (GENBANK PROTEIN ID YP\_184940.1); Size: 55AA.

MLEESREQLSVEEYETFNRFDNQEFDFERELTQDPYSKVYLYSIEDHIRTYKIEK

FRAGMENTS

&gt;SEQ ID NO:6

CTAGAATCTAGAGAGCAATTATCAGTCGAAGAATACGAAACATTCTTTAACAGATTTGATAATCAAGAATTTGATTTCGA  
 ACGTGAATTGACACAAGATCCATATTCAAAAGTATACTTATACAGTATAGAAGACCATATCAGAACATATAAGATAGAGA  
 AATAA

&gt;SEQ ID NO:7; localization in full length SACOL0029: 2-55; size: 54 AA.

LEESREQLSVEEYETFNRFDNQEFDFERELTQDPYSKVYLYSIEDHIRTYKIEK

VARIANT

>SEQ ID NO:8; Comprising a SACOL0029 fragment localized in the full length SACOL0029: 2-55. The polyhistidine is double underlined.

MHHHHHLEESREQLSVEEYETFNRFDNQEFDFERELTQDPYSKVYLYSIEDHIRTYKIEK

>SEQ ID NO:9; Comprising a SACOL0029 fragment localized in the full length SACOL0029: 2-55. The RGS-polyhistidine-GS tag is double underlined.

MRGSHHHHHGSLEESREQLSVEEYETFNRFDNQEFDFERELTQDPYSKVYLYSIEDHIRTYKIEK

FIG. 21A

II. SACOL0720FULL LENGTHPOLYNUCLEOTIDE

>SEQ ID NO: 10 (GENBANK GENE ID 3236600).

ATGACCTTTAACGAGATAATATTTAAAAATTTCCGTCAAAATTTATCACATTATGCCATCTATCTTTTTTCGTTAATTAC  
 GAGTGTAGTATTGTATTTTAGCTTTGTAGCATTAAAATACGCTCATAAACTAAACATGACAGAGTCATATCCAATTATAA  
 AGGAAGGCTCACAAAGTCGGAAGCTACTTTCTATTTTTCATCATAATTGCATTTTGTATATGCCAATGTGTTATTTATT  
 AAACGACGAAGTTATGAGCTTGCATTATATCAAACATTAGGTTTATCTAAATTCAACATTATTTATATACTAATGCTCGA  
 ACAATTACTAATATTTATAATTACGGCAATATTAGGTATTATTATTGGTATTTTGGTTCGAAACTGTTATTAATGATTG  
 TCTTTACATTATTAGGAATTAAAGAAAAGGTTCCAATTATTTTATAGTTTGGAGGCGGTATTTGAAACATTAATGTTAATC  
 GGTGTCGCTTATTTTAAACATCTGCTCAAAATTTATATTAGTGTCAAACAATCTATTTACAGATGTCAAAAGAATAA  
 CCAGGTTAAAGAAACAAATCATAATAAAATTACATTTGAAGAGGTTGTTTTAGGCATCTTAGGTATAGTATTGATTACCA  
 CAGGATACTATCTATCTTTGAACATTGTTCAATATTATGATTCTATCGGTACACTTATGTTTATTTTATTGTCAACTGTG  
 ATTGGGGCATACTTATTTTAAAGCTCTGTTTCTCTAGTTTTTAAATGGTGAAGAAGTTTAGAAAAGGTGTTATAAG  
 TGTAATGATGTCATGTTCTCATCATCTATTATGTATCGTATTAAGAAAAATGCTTTTTCACCTACGGTCATGGCAATCA  
 TTTTCAGCGATTACTGTTTCAGTTCTTTGCTTTGCTGCTATAAGTAGAGCGTCTTATCAAGTGAAATAAAATATACTGCA  
 CCACACGACGTTACAATTAAAGACCAACAAAAGCTAATCAATTAGCAAGTGAATTAACAATCAAAAAATTCCTCATT  
 TTATAATTATAAGAAGTAATTCATACGAAATTGTATAAGATAATTTATTTGATGTAAAGCGAAAGAACCATAACAATG  
 TAACAATTACTAGTGATAAATACATCCCTAATACTGATTTGAAACGTGGGCAAGCTGATTTATTTGTAGCGGAAGGTTCT  
 ATCAAAGATTTAGTGAAACATAAGAAGCATGTTAAGGCAATTATAGGAACGAAAAACATCATGTTAATATTAAGTTACG  
 TAAAGATATTAATAAAATCTATTTTATGACAGATGTTGATTTAGGTGGACCAACGTTTGTCTTAAATGACAAAGACTATC  
 AAGAAATAAGAAAGTATACAAAGGCAAAGCATATCGTCTCTCAATTTGGATTTCGATTTGAAACATAAAAAAGATGCTTTA  
 GCATTAGAAAAAGCGAAAAATAAAGTTGATAAATCTATTGAAACAAGAAGTGAAGCGATAAGCTCAATATCAAGTTTAAC  
 CGGAATATTATTATTTGTAACATCATTTTGGTATTACATTCCTGATTGCTGTATGTTGCATTATATACATAAAGCAAA  
 TAGATGAAACCGAAGATGAGTTAGAGAATTATAGTATTTGAGAAAGCTTGGATTTACACAAAAAGATATGGCAAGGGGA  
 CTAAAGTTTAAATATGTTTAATTTTGGGTACCTTTAGTTATTGCACATACATGCATATTTTACATCATTAGCATA  
 TATGAAATTAATGGGTACAACGAATCAAATACCGGTTTTCATAGTAATGGGATTATACATTTGTATGTATGCTGTTTTTG  
 CAGTGACGGCTTATAATCATTCGAAGCGAACATTAGACATTCCATATAA

POLYPEPTIDE

> SEQ ID NO: 11 (GENBANK PROTEIN ID YP\_185601.1); Size: 629AA.

MTFNEIIFKNFRQNLSHYAIYLFSLITSVVLVYFSFVALKYAHKLNMTESYPIIKEGSQVGSYFLFFIIIAFLLYANVLF  
 KRRSYELALYQTLGLSKFNIIYILMLEQLLIFIITAILGIIIGIFGSKLLMIVFTLLGIKEKVPIIFSLRAVFETLMLI  
 GVAYFLTSAQNFIILVFKQSIQMSKNNQVKETNHNKITFEVVVLGILGIVLITGYYLSLNIVQYYDSIGTLMFILLSTV  
 IGAYLFFKSSVSLVFKMVKKFRKGVISVNDVMFSSSIMYRIKKNAFSLTVMAIISAITVSVLCFAAISRASLSSEIKYTA  
 PHDVTIKDQOKANQLASELNNQKIPHFYNYKEVIHTKLYKDNLFVKAKEPYNVTITSDKYIPNTDLKRGQADLFVAEGS  
 IKDLVKHKKHKGKAIIGTKKHHVNIKLKDKINKIYFMTDVLGGPTFVLNDKDYQEIIRKYTKAKHIVSQFGFDLKHKKDAL  
 ALEKAKNKVDKSIETRSEAISSISLGTGILFVTSFLGITFLIAVCCIIYIKQIDETEDELLENYSILRKLGTQKDMARG  
 LKFKIMFNFLPLVIALSHAYFTSLAYMKLMGTNQIPVFIVMGLYICMYAVFAVTAYNHSKRTIRHSI

FIG. 21B

FRAGMENTS

>SEQ ID NO : 12 ; localization in full length SACOL0720: 309-508; size: 200 AA.

RASLSSEIKYTAPHDVTIKDQQKANQLASELNNQKIPHFYNYKEVIHTKLYKDNLFDVKAKEPYNVTITSDKYIPNTDLKRGQ  
QADLFVAEGSIKDLVKHKKHKGKAIIGTKKHHVNIKLKDKINKIYFMTDVDLGGPTFVLNDKDYQEIRKYTKAKHIVSQFGFDL  
KHKKDALALEKAKNKVDKSIETRSEAIISSISLTG

>SEQ ID NO : 13; localization in full length SACOL0720: 310-508; size: 199 AA.

ASLSSEIKYTAPHDVTIKDQQKANQLASELNNQKIPHFYNYKEVIHTKLYKDNLFDVKAKEPYNVTITSDKYIPNTDLKRGQ  
ADLFVAEGSIKDLVKHKKHKGKAIIGTKKHHVNIKLKDKINKIYFMTDVDLGGPTFVLNDKDYQEIRKYTKAKHIVSQFGFDL  
KHKKDALALEKAKNKVDKSIETRSEAIISSISLTG

>SEQ ID NO : 14 ; localization in full length SACOL0720: 310-455; size: 146 AA.

ASLSSEIKYTAPHDVTIKDQQKANQLASELNNQKIPHFYNYKEVIHTKLYKDNLFDVKAKEPYNVTITSDKYIPNTDLKRGQ  
ADLFVAEGSIKDLVKHKKHKGKAIIGTKKHHVNIKLKDKINKIYFMTDVDLGGPTFVLNDKDYQE

>SEQ ID NO : 15 ; localization in full length SACOL0720: 428-481; size: 54 AA.

KDINKIYFMTDVDLGGPTFVLNDKDYQEIRKYTKAKHIVSQFGFDLKHKKDALA

>SEQ ID NO : 16 ; localization in full length SACOL0720: 428-452; size: 25 AA.

KDINKIYFMTDVDLGGPTFVLNDKD

>SEQ ID NO : 17 ; localization in full length SACOL0720: 428-453; size: 26 AA.

KDINKIYFMTDVDLGGPTFVLNDKDY

>SEQ ID NO : 18 ; localization in full length SACOL0720: 467-481; size: 15 AA.

SQFGFDLKHKKDALA

>SEQ ID NO : 19 ; localization in full length SACOL0720: 428-450; size: 23 AA.

KDINKIYFMTDVDLGGPTFVLND

>SEQ ID NO : 20 ; localization in full length SACOL0720: 463-481; size: 19 AA.

KHIVSQFGFDLKHKKDALA

>SEQ ID NO : 21 ; localization in full length SACOL0720: 468-481; size: 14 AA.

QFGFDLKHKKDALA

>SEQ ID NO : 22 ; localization in full length SACOL0720: 325-337; size: 13 AA.

**FIG. 21C**

TIKDOOKANQLAS

>SEQ ID NO : 23 ; localization in full length SACOL0720: 428-441; size: 14 AA.

KDINKIYFMTDVDL

>SEQ ID NO : 24 ; localization in full length SACOL0720: 438-450; size: 13 AA.

DVDLGGPTFVLND

#### VARIANT

>SEQ ID NO: 25 with polyhistidine in N-terminus; Comprises fragment localized in full length SACOL0720 at positions 310-455. The polyhistidine is double underlined.

MHHHHHHASLSSEIKYTAPHDVTIKDOOKANQLASELNNQKIPHFYNYKEVIHTKLYKDNLFVDVKAKEPYNVTITSDKYIPNTDLKRGQADLFVAEGSIKDLVKHKKHGKAIIGTKKHHVNIKLRKDINKIYFMTDVDLGGPTFVLNDKDYQE

>SEQ ID NO: 26 with polyhistidine in N-terminus; Comprises fragment localized in full length SACOL0720 at positions 310-455. The RGS-polyhistidine-GS tag is double underlined.

MRGSHHHHHHGSASLSSEIKYTAPHDVTIKDOOKANQLASELNNQKIPHFYNYKEVIHTKLYKDNLFVDVKAKEPYNVTITSDKYIPNTDLKRGQADLFVAEGSIKDLVKHKKHGKAIIGTKKHHVNIKLRKDINKIYFMTDVDLGGPTFVLNDKDYQE

>SEQ ID NO: 27 Comprises fragments localized in full length SACOL0720 at positions 428-450 and 463-481. Size: 50 AA. The underlined sequence is variant vs. native sequence. The remaining portions are native.

KDINKIYFMTDVDLGGPTFVLNDKDYERKYKHIVSQEGFDLKHKKDALA

### III. SACOL0442

#### FULL LENGTH

#### POLYNUCLEOTIDE

>SEQ ID NO:28 (GENBANK GENE ID 3236485).

ATGTTCAAAAAATATGACTCAAAAAATCAATCGTATTAAAAATCTATTCTATCGCTAGGTATCATCTATGGGGGAACATTGGAATATATCCAAAAGCAGACGCGTCAACACAAAATTCCTCAAGTGTACAAGATAAACAATTACAAAAGTTGAAGAAGTACCAAATAATTCAGAAAAGCCTTTGGTTAAAAAATTTACGATAGATACAGCAAGGATACAATAAATGGAAAATCTAATAAATCTAGGAATTGGGTTATTTCAGAGAGACCTTTAAATGAAAACCAAGTTCGTATACATTTAGAAGGAACATACACAGTTGCTGGCAGAGTGTATACACCTAAGAGGAATATTACTCTTAATAAAGAAGTTGTCACTTTAAAAGAATTGGATCATATCATAAGATTTGCTCATATTTCTATGGCTTGTATATGGGAGAACATTGCGCTAAAGGTAACATCGTCATAAATACAAAAGATGGTGGTAAATATACATTAGAGTCGCATAAAGAGCTACAAAAGATAGGGAAAATGTAAAAATTAATACAGCCGATATAAAAAATGTAACCTTTCAAACCTTGTAAGTGTAAATGACATTGAACAAGTTTGA

#### POLYPEPTIDE

>SEQ ID NO: 29 (GENBANK PROTEIN ID YP\_185332.1); Size:203AA.

MFKKYDSKNSIVLKSILSLGIYGGTFGIYPKADASTQNSSSVQDKQLQKVEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNIVYSE RPLNENQVRIHLEGTYTAVGRVYTPKRNITLNKEVVTLKELDHIIRFAHISYGLYMGEHLPKGNIVINTKDGGKYTLESHKELOKDREN VKINTADIKNVTFKLKSVNDIEQV

**FIG. 21D**

FRAGMENTS

>SEQ ID NO: 30; localization in full length SACOL0442: 36-203; Size: 168 AA.

STQNSSSVQDKQLQKVEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIHLEGTYTVAGRVYTPKRNITLNKEVVTLKELDHIIRFAHISYGLYMGEHLPKGNIVINTKDGGKYTLESHKELQKDRENVKINTADIKNVTFKLVKSVNDIEQV

>SEQ ID NO: 31; localization in full length SACOL0442: 44-159; Size: 116 AA.

QDKQLQKVEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIHLEGTYTVAGRVYTPKRNITLNKEVVTLKELDHIIRFAHISYGLYMGEHLPKGNIVINTK

>SEQ ID NO: 33; localization in full length SACOL0442: 45-203; Size: 159 AA.

DKQLQKVEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIHLEGTYTVAGRVYTPKRNITLNKEVVTLKELDHIIRFAHISYGLYMGEHLPKGNIVINTKDGGKYTLESHKELQKDRENVKINTADIKNVTFKLVKSVNDIEQV

>SEQ ID NO: 34; localization in full length SACOL0442: 72-85; Size: 14 AA.

KDTINGKSNKSRNW

>SEQ ID NO: 1; localization in full length SACOL0442: 159-173; Size: 15 AA.

KDGGKYTLESHKELQ

VARIANTS

>SEQ ID NO: 35; Comprises fragment localized in full length SACOL0442 at positions 45-203. The polyhistidine is double underlined.

MHHHHHDKQLQKVEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIHLEGTYTVAGRVYTPKRNITLNKEVVTLKELDHIIRFAHISYGLYMGEHLPKGNIVINTKDGGKYTLESHKELQKDRENVKINTADIKNVTFKLVKSVNDIEQV

>SEQ ID NO: 36; Comprises fragment localized in full length SACOL0442 at positions 45-203. The RGS-polyhistidine-GS tag is double underlined.

MRGSHHHHHGSDKQLQKVEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIHLEGTYTVAGRVYTPKRNITLNKEVVTLKELDHIIRFAHISYGLYMGEHLPKGNIVINTKDGGKYTLESHKELQKDRENVKINTADIKNVTFKLVKSVNDIEQV

**FIG. 21E**

IV. SACOL1867FULL LENGTHPOLYNUCLEOTIDE

>SEQ ID NO: 37 (GENBANK GENE ID 3236101).

ATGAATAAAAATATAGTCATTAAAAGCATGGCAGCATTAGCCATTCTAACCTCAGTAACTGGAATAAATGCTGCAGTCGT  
TGAAGAGACACAAACAAATAGCAAATGCAGAGAAGAATGTTACGCAAGTTAAAGATACAAATATTTTCCATATAATGGCG  
TCGTTTCATTTAAAGATGCGACAGGTTTTGTAAATTGGAAAAAATACAATTATCACCAATAAACATGTATCAAAAGATTAT  
AAAGTTGGCGATAGAATTACTGCCCATCCAAACGGTGACAAAGGAAATGGTGGTATATATAAAATTTAAAGCATTTCTGA  
TTATCCGGGTGATGAAGACATCTCTGTCTATGAATATTGAAGAACAAGCAGTCGAACGTGGACCAAAGGCTTAATTTTA  
ATGAAAATGTCCAAGCATTTCAATTTTGCAGAAAGATGCTAAAGTTGATGACAAAATTTAAAGTTATTGGTTACCCATTACCT  
GCTCAAAATAGTTTAAACAGTTTGAATCTACAGGAAGTATAAAAAGAATCAAAGACAATATTTTAAATTTTATGTCATA  
CATTGAACCCGGGAATTCAGGATCACCAGTTCTAAATTTCTACAATGAGGTCATAGGTGTGGTGTATGGCGGTATTGGAA  
AAATTGGTTCTGAATATAATGGTGCCGTATACTTTACGCCTCAAATCAAAGATTTTATTCAAAGCACATTGAACAATAA

POLYPEPTIDE

>SEQ ID NO: 38 (GENBANK PROTEIN ID YP\_186695.1); Size: 239 AA.

**MNKNIVIKSMAALAILTSVTGINAAVVEETQQIANAEKNVTQVKD**TNIFPYNGVVSFKDATGEVIGKNTIITNKHVSKDY  
KVGDRITAHPNGDKGNGGIYKIKSISDYPGDEDISVMNIEEQAVERGPKGFNFENVQAFNFAKDAKVDDKIKVIGYPLP  
AQNSFKQFESTGTIKRIKDNILNFDAYIEPGNSGSPVLNSNNEVIGVVYGGIGKIGSEYNGAVYFTPQIKDFIQKHIEQ

FRAGMENTS

>SEQ ID NO: 39; localization in full length SACOL1867: 41-239; Size: 199 AA.

TQVKD**TNIFPYNGVVSFKDATGEVIGKNTIITNKHVSKDYKVGDRITAHPNGDKGNGGIYKIKSISDYPGDEDISVMNIE**  
EQAVERGPKGFNFENVQAFNFAKDAKVDDKIKVIGYPLPAQNSFKQFESTGTIKRIKDNILNFDAYIEPGNSGSPVLNS  
NNEVIGVVYGGIGKIGSEYNGAVYFTPQIKDFIQKHIEQ

VARIANT

>SEQ ID NO: 40; Comprises fragment localized in full length SACOL1867 at positions 41-239.  
Polyhistidine double underlined.

MHHHHHTQVKDTNIFPYNGVVSFKDATGEVIGKNTIITNKHVSKDYKVGDRITAHPNGDKGNGGIYKIKSISDYPGDE  
ISVMNIEEQAVERGPKGFNFENVQAFNFAKDAKVDDKIKVIGYPLPAQNSFKQFESTGTIKRIKDNILNFDAYIEPGNS  
GSPVLNSNNEVIGVVYGGIGKIGSEYNGAVYFTPQIKDFIQKHIEQ

>SEQ ID NO: 41; Comprises fragment localized in full length SACOL1867 at positions 41-239. The  
RGS-polyhistidine-GS tag is double underlined.

MRGSHHHHHGSTQVKDTNIFPYNGVVSFKDATGEVIGKNTIITNKHVSKDYKVGDRITAHPNGDKGNGGIYKIKSISD  
PGDEDISVMNIEEQAVERGPKGFNFENVQAFNFAKDAKVDDKIKVIGYPLPAQNSFKQFESTGTIKRIKDNILNFDAYI  
EPGNSGSPVLNSNNEVIGVVYGGIGKIGSEYNGAVYFTPQIKDFIQKHIEQ

**FIG. 21F**

V. SACOL1912FULL LENGTHPOLYNUCLEOTIDE

>SEQ ID NO: 42 (GENBANK GENE ID 3236086).

ATGGCAATGAAC TTTAAAGTCTTTGACAATAGTCAACTTGTAGCAGAATATGCTGCTGATATTATTAGAAAGCAATTTAA  
CAATAATCCTACTACAATTGCAGGTTTTTCATTTAGATACAGATCAAGCGCCAGTTCTAGATGAATTAAGAAAAATGTTG  
AAAAACATGCTGTTGATTTTAGCCAAATAAATATTTTAGATTATGACGATAAAAAATCATATTTTGAAGCGTTAGGTGTA  
CCAGCAGGTCAAGTTTATCCAATTGCTTATGAAAAAGATGCAATCGAATTAATCGCTGATAAGATTAAACTAAAGAAAA  
TAAAGGGAAATTAACATTACAAGTTGTTTCTATCGATGAGCAAGGTAAGTTAAATGTTAGTATTCGTCAGGACTAATGG  
AAGCAAGAGAAATTTCTTAGTAGTGACAGGTGCTAATAAACGAGATGTAGTTGAAAAATTATATCAAGAAAAATGGTAAA  
ACAAGCTTCGAACCGAGCCGATTTAAAGCACATAGAATGGTAAATGTTATTCTTGATAAAGAAGCGGCTGCAGGTTTACC  
TGAAGATGTTAAAGCTTACTTTACGTCACGCTTGCCTAA

POLYPEPTIDE

>SEQ ID NO: 43 (GENBANK PROTEIN ID YP\_186737.1); Size: 199AA.

MAMNFKVFDNSQLVAEYAADIIRKQFNNNP<sup>1</sup>TTIAGFHLDTDQAPVLDELKKNVEKHAVDFSQINILDYDDKKS<sup>2</sup>YFEALGV  
PAGQVYPIAYEKDAIELIADKIKTKENKGKLT<sup>3</sup>LQVVSIDEQGKLNVSIRQGLMEAREIFLVVTGANKRDVVEKLYQENGK  
TSFEPADLKAHRMVNVILDKEAAAGLPEDVKAYFTSRFA

VARIANT

>SEQ ID NO: 44 ; Comprises full length SACOL1912. The polyhistidine is double underlined.

MHHHHHHMAMNFKVFDNSQLVAEYAADIIRKQFNNNP<sup>1</sup>TTIAGFHLDTDQAPVLDELKKNVEKHAVDFSQINILDYDDKKS  
YFEALGV<sup>2</sup>PAGQVYPIAYEKDAIELIADKIKTKENKGKLT<sup>3</sup>LQVVSIDEQGKLNVSIRQGLMEAREIFLVVTGANKRDVVEK  
LYQENGKTSFEPADLKAHRMVNVILDKEAAAGLPEDVKAYFTSRFA

VI. SACOL2385FULL LENGTHPOLYNUCLEOTIDE

> SEQ ID NO: 49 (GENBANK GENE ID 3238646).

ATGATATTGAAC TCAATCAATTCGAGAATCAAACTTTTTTAAACGGTAATCCAAGTGATACATTTAAAGATTTAGGTAA  
ACAAGTATTTAATTACTTTTCAACACCTTCATTTGTAACGAATATATATGAAACAGACGAATTATATTACTTAGAAGCTG  
AACTAGCAGGTGTAAATAAAGAAGATATTAGTATCGATTTCAATAATAATACGCTCACTATTCAAGCTACTAGAAGCGCA  
AAATACAAATCTGAACAAC TCAATTTAGATGAGCGTAAC TCGAATCATTAATGCGTCAATTTGATTTTGAAGCTGTTGA  
TAAGCAACATATTACTGCTAGTTTGGAAATGGGTATTAACCATACCTTGCCTAAATCAAACCAAGCAATGAAACTA  
CTTCATCAACATCTATTCCAATTTTCATAG

**FIG. 21G**

POLYPEPTIDE

> SEQ ID NO: 50 (GENBANK PROTEIN ID YP\_187189.1); Size: 93AA.

MILNFNQFENQNF**FNGNPSDTEFKDLGKQ**VFNFYSTPSFVTNIYETDELYYLEAELAGV**NKEDISIDFNNTLT**IQATRSA  
KYKSEQLILDERN

VARIANT

>SEQ ID NO: 51; Comprises full length SACOL2385.

MHHHHH**MILNFNQFENQNF****FNGNPSDTEFKDLGKQ**VFNFYSTPSFVTNIYETDELYYLEAELAGV**NKEDISIDFNNTLT**  
**I**QATRS**AKYKSEQLILDERN**

VII. FUSIONS

>SACOL0029-1867 (1)

POLYNUCLEOTIDE

>SEQ ID NO: 54

CTAGAATCTAGAGAGCAATTATCAGTCGAAGAATACGAAACATTCTTTAACAGATTGATAATCAAGAATTTGATTTCGA  
ACGTGAATTGACACAAGATCCATATTCAAAGTATACTTATACAGTATAGAAGACCATATCAGAACATATAAGATAGAGA  
AAGGAGGTGGCGGTTTCAGGAGGTGGAGGATCTGGAGGCGGTGGATCA**ACGCAAGTTAAAGATACAAATATTTTCCATAT**  
**AATGGCGTCGTTTCATTTAAAGATGCGACAGGTTTTGTAATTGGAAAAATACAATTATCACCAATAAACATGTATCAA**  
**AGATTATAAAGTTGGCGATAGAATTACTGCCCATCCAAACGGTGACAAAGGAAATGGTGGTATATATAAAATTTAAAGCA**  
**TTTCTGATTATCCGGGTGATGAAGACATCTCTGTCATGAATATTGAAGAACAAGCAGTCGAACGTGGACCAAAGGCTTT**  
**AATTTTAATGAAATGTCCAAGCATTCAATTTTGCGAAAGATGCTAAAGTTGATGACAAAATTAAAGTTATTGGTTACCC**  
**ATTACCTGCTCAAAATAGTTTTAAACAGTTTGAATCTACAGGAACATAAAAAAGAAATCAAAGACAATATTTTAAATTTG**  
**ATGCATACATTGAACCCGGAATTCAGGATCACCAAGTTCTAAATTCTAACAATGAGGTCATAGGTGTGGTGTATGGCGGT**  
**ATTGGAAAAATTGGTTCTGAATATAATGGTGCCGTATACTTTACGCCTCAAATCAAAGATTTTATTCAAAGCACATTGA**  
**ACAATAA**

POLYPEPTIDE

>SEQ ID NO: 55; Size: 268AA.

**LESREQLSVEEYET****FNRFDN****QEFDFERELTQDP****YSKVYLYSIEDHIRTYKIEKGGGSGGGSGGGGS****TQVKDTNIFPY**  
**NGVVSFKDATG****FVIGKNTIITNKHVSKDYKVGDRITAHPNGDKNGGIYKIKSISDYPGDEDISVMNIEEQ****AVERGPKGF**  
**NFENVQAFNFAKDAKVDDKIKVIGYPLPAQNSFKQ****FESTGTIKRIKDNI****LNFDAYIEPGNSGSPVLNSNNEVIGVVYGG**  
**IGKIGSEYNGAVYFTPQIKDFIQKHIEQ**

FIG. 21H

>SACOL0029-1867 (2)

POLYNUCLEOTIDE

>SEQ ID NO: 56

ATGCACCACCACCACCACCACCCTGGAATCCCGTGAACAACGTGTCGGTGAAGAATACGAAACCTTCTTTAACCGCTTTGATAACCAAGA  
ATTTGATTTTCGAACGTGAACGTGACCCAGGATCCGTATTCTAAAGTGTATCTGTACAGTATCGAAGATCATATTGCACGTACAAAATCG  
AAAAAGCGGGTGGCGGTTCTGGCGGTGGCGGTAGTGGCGGTGGCGGTAGCACCCAGGTGAAAGATACGAATATCTTCCGTATAACGGC  
GTGGTTTCTTTTAAAGATGCGACCGGCTTCGTTATCGGTAAAAACACCATCATCACGAACAAACATGTGAGCAAAGATTACAAAGTTGG  
CGATCGTATTACCGCCACCCGAATGGCGATAAGGGTAACGGCGGTATCTACAAAATCAAAGCATCTCTGATTACCGGGTGATGAAG  
ATATCAGCGTGATGAATATTGAAGAACAGGCAGTTGAACGCGGCCGAAAGGTTTAACTTCAATGAAAACGTTTCAGGCGTTTAATTC  
GCGAAAGATGCCAAAGTGGATGATAAAATCAAAGTTATTGGCTATCCGCTGCCGGCCCGAAGCAGCTTTAAACAGTTCGAATCTACCGG  
TACGATCAAACGTATCAAAGATAACATCCTGAACCTTCGATGCATATATTGAACCGGGCAATAGTGGTAGCCCGGTGCTGAACAGTAACA  
ATGAAGTTATTGGTGTGGTTTATGGCGGTATCGGCAAAATGGTAGCGAATACAACGGTGCTGTGTATTTACCGCGCAGATCAAAGAC  
TTCATCCAGAAACATATCGAACA

POLYPEPTIDE

>SEQ ID NO: 57; Size: 275AA. The polyhistidine is double underlined

MHHHHHHHLESREQLSVEEYETFNRFNEEFFERELTQDPYSKVLYSIEDHIRTYKIEKGGGGSGGGSGGGGSTQVKDTNIFPYNG  
VVSFKDATGFVIGKNTIITNKHVSKDYKVGDRITAHPNGDKNGGIYKIKSISDYPGDEDISVMNIEEQAVERGPKGFNFENVQAFNF  
AKDAKVDKIKVIGYPLPAQNSFKQFESTGTIKRIKDNILNFDAYIEPGNSGSPVLNSNNEVIGVYGGIGKIGSEYNGAVYFTPQIKD  
FIQKHIEQ

>SEQ ID NO: 58; Size: 280AA. The RGS-polyhistidine-GS tag is double underlined.

MRGSHHHHHHGSLESREQLSVEEYETFNRFNEEFFERELTQDPYSKVLYSIEDHIRTYKIEKGGGGSGGGSGGGGSTQVKDT  
NIFPYNGVVSFKDATGFVIGKNTIITNKHVSKDYKVGDRITAHPNGDKNGGIYKIKSISDYPGDEDISVMNIEEQAVERGPKGFNFNE  
NVQAFNFAKDAKVDKIKVIGYPLPAQNSFKQFESTGTIKRIKDNILNFDAYIEPGNSGSPVLNSNNEVIGVYGGIGKIGSEYNGAVY  
FTPQIKDFIQKHIEQ

>SACOL0720-720

POLYPEPTIDE

>SEQ ID NO: 27.

KDINKIYFMTDVLGGPTFVLNDKDYERKYKKHIVSQFGFDLKHKKDALA

>SACOL00442-720

POLYPEPTIDE

>SEQ ID NO: 3.

KDGGKYTLSEHKELQEAAAKEAAAKKDINKIYFMTDVLGGPTFVLND

**FIG. 211**

VIII. LINKERS

Used in exemplified fusion SACOL0029-1867.

POLYNUCLEOTIDE

>SEQ ID NO: 59

GGAGGTGGCGGTTTCAGGAGGTGGAGGATCTGGAGGCGGTGGATCA

POLYPEPTIDE

>SEQ ID NO: 60.

GGGGSGGGSGGGGS

POLYPEPTIDE Used in exemplified fusion SACOL00720-720

>SEQ ID NO: 61.

**ERKYK**

POLYPEPTIDE Used in exemplified fusion SACOL00442-720

>SEQ ID NO: 62.

**EAAAKEAAAK**

Other exemplary linker polypeptides

>SEQ ID NO: 63.

EAAAK

>SEQ ID NO: 64.

EAAAKEAAAKEAAAK

>SEQ ID NO: 65.

ERKYKERKYK

>SEQ ID NO: 66.

ERKYKERKYKERKYK

>SEQ ID NO: 67.

GGGS

>SEQ ID NO: 68.

GGGGSGGGGS

>SEQ ID NO: 69.

XPXPXP wherein x may be any amino acid.

>SEQ ID NO: 70.

XPXPXPXPXP, wherein x may be any amino acid.

**FIG. 21J**

**I- SACOL0442 : Multiple polynucleotide sequences alignment**

```

MW0345 (MW2)                ATGTTCAAAAAAATGACTCGAAAAATTC AATTCTATTA AATCTATTCT 50
SAS0347 (MSSA476)           ATGTTCAAAAAAATGACTCGAAAAATTC AATTCTATTA AATCTATTCT 50
SACOL0442 (COL)              ATGTTCAAAAAAATGACTCGAAAAATTC AATCGTATTAA AATCTATTCT 50
SAOUHSC_00354 (NCTC8325)    ATGTTCAAAAAAATGACTCGAAAAATTC AATCGTATTAA AATCTATTCT 50
NWMN_0362 (Newman)          ATGTTCAAAAAAATGACTCGAAAAATTC AATCGTATTAA AATCTATTCT 50
SAUSA300_0370 (USA300-FPR3757) ATGTTCAAAAAAATGACTCGAAAAATTC AATCGTATTAA AATCTATTCT 50
SaurJH1_0429 (JH1)          ATGTTCAAAAAAATGACTCGAAAAATTC AATCGTATTAA AATCTATTCT 50
SAHV_0367 (Mu3)             ATGTTCAAAAAAATGACTCGAAAAATTC AATCGTATTAA AATCTATTCT 50
SaurJH9_0419 (JH9)          ATGTTCAAAAAAATGACTCGAAAAATTC AATCGTATTAA AATCTATTCT 50
SAV0370 (Mu50)              ATGTTCAAAAAAATGACTCGAAAAATTC AATCGTATTAA AATCTATTCT 50
SA0357 (N315)               ATGTTCAAAAAAATGACTCGAAAAATTC AATCGTATTAA AATCTATTCT 50
SAB0321 (RF122)             ATGTTCAAAAAAATGACTCGAAAAATTC AATCGTATTAA AATCTATTCT 50
*****

MW0345 (MW2)                ATCGTAGGTATCATCTATGGGGGAACATT TGG AATATATCCAAAAGCAG 100
SAS0347 (MSSA476)           ATCGTAGGTATCATCTATGGGGGAACATT TGG AATATATCCAAAAGCAG 100
SACOL0442 (COL)              ATCGTAGGTATCATCTATGGGGGAACATT TGG AATATATCCAAAAGCAG 100
SAOUHSC_00354 (NCTC8325)    ATCGTAGGTATCATCTATGGGGGAACATT TGG AATATATCCAAAAGCAG 100
NWMN_0362 (Newman)          ATCGTAGGTATCATCTATGGGGGAACATT TGG AATATATCCAAAAGCAG 100
SAUSA300_0370 (USA300-FPR3757) ATCGTAGGTATCATCTATGGGGGAACATT TGG AATATATCCAAAAGCAG 100
SaurJH1_0429 (JH1)          ATCGTAGGTATCATCTATGGGGGAACATT TGG AATATATCCAAAAGCAG 100
SAHV_0367 (Mu3)             ATCGTAGGTATCATCTATGGGGGAACATT TGG AATATATCCAAAAGCAG 100
SaurJH9_0419 (JH9)          ATCGTAGGTATCATCTATGGGGGAACATT TGG AATATATCCAAAAGCAG 100
SAV0370 (Mu50)              ATCGTAGGTATCATCTATGGGGGAACATT TGG AATATATCCAAAAGCAG 100
SA0357 (N315)               ATCGTAGGTATCATCTATGGGGGAACATT TGG AATATATCCAAAAGCAG 100
SAB0321 (RF122)             ATCGTAGGTATCATCTATGGGGGAACATT TGG AATATATCCAAAAGCAG 100
*****

MW0345 (MW2)                ACGCGTCAACACAAAAATTCCTCAAGTGTACAAGATAAACAATTCCAAAAA 150
SAS0347 (MSSA476)           ACGCGTCAACACAAAAATTCCTCAAGTGTACAAGATAAACAATTCCAAAAA 150
SACOL0442 (COL)              ACGCGTCAACACAAAAATTCCTCAAGTGTACAAGATAAACAATTACAAAAA 150
SAOUHSC_00354 (NCTC8325)    ACGCGTCAACACAAAAATTCCTCAAGTGTACAAGATAAACAATTACAAAAA 150
NWMN_0362 (Newman)          ACGCGTCAACACAAAAATTCCTCAAGTGTACAAGATAAACAATTACAAAAA 150
SAUSA300_0370 (USA300-FPR3757) ACGCGTCAACACAAAAATTCCTCAAGTGTACAAGATAAACAATTACAAAAA 150
SaurJH1_0429 (JH1)          ACGCGTCAACACAAAAATTCCTCAAGTGTACAAGATAAACAATTACAAAAA 150
SAHV_0367 (Mu3)             ACGCGTCAACACAAAAATTCCTCAAGTGTACAAGATAAACAATTACAAAAA 150
SaurJH9_0419 (JH9)          ACGCGTCAACACAAAAATTCCTCAAGTGTACAAGATAAACAATTACAAAAA 150
SAV0370 (Mu50)              ACGCGTCAACACAAAAATTCCTCAAGTGTACAAGATAAACAATTACAAAAA 150
SA0357 (N315)               ACGCGTCAACACAAAAATTCCTCAAGTGTACAAGATAAACAATTACAAAAA 150
SAB0321 (RF122)             ACGCGTCAACACAAAAATTCCTCAAGTGTACAAGATAAACAATTCCAAAAA 150
*****

MW0345 (MW2)                GTTGAAGAAGTACCAAATAATTCAGAAAAAGCTTTGGTTAAAAAACTTTA 200
SAS0347 (MSSA476)           GTTGAAGAAGTACCAAATAATTCAGAAAAAGCTTTGGTTAAAAAACTTTA 200
SACOL0442 (COL)              GTTGAAGAAGTACCAAATAATTCAGAAAAAGCTTTGGTTAAAAAACTTTA 200
SAOUHSC_00354 (NCTC8325)    GTTGAAGAAGTACCAAATAATTCAGAAAAAGCTTTGGTTAAAAAACTTTA 200
NWMN_0362 (Newman)          GTTGAAGAAGTACCAAATAATTCAGAAAAAGCTTTGGTTAAAAAACTTTA 200
SAUSA300_0370 (USA300-FPR3757) GTTGAAGAAGTACCAAATAATTCAGAAAAAGCTTTGGTTAAAAAACTTTA 200
SaurJH1_0429 (JH1)          GTTGAAGAAGTACCAAATAATTCAGAAAAAGCTTTGGTTAAAAAACTTTA 200
SAHV_0367 (Mu3)             GTTGAAGAAGTACCAAATAATTCAGAAAAAGCTTTGGTTAAAAAACTTTA 200
SaurJH9_0419 (JH9)          GTTGAAGAAGTACCAAATAATTCAGAAAAAGCTTTGGTTAAAAAACTTTA 200
SAV0370 (Mu50)              GTTGAAGAAGTACCAAATAATTCAGAAAAAGCTTTGGTTAAAAAACTTTA 200
SA0357 (N315)               GTTGAAGAAGTACCAAATAATTCAGAAAAAGCTTTGGTTAAAAAACTTTA 200
SAB0321 (RF122)             GTTGAAGAAGTACCAAATAATTCAGAAAAAGCTTTGGTTAAAAAACTGTA 200
*****

```

**FIG. 22A**

```
MW0345 (MW2)
SAS0347 (MSSA476)
SACOL0442 (COL)
SAOUHSC_00354 (NCTC8325)
NWMN_0362 (Newman)
SAUSA300_0370 (USA300-FPR3757)
SaurJH1_0429 (JH1)
SAHV_0367 (Mu3)
SaurJH9_0419 (JH9)
SAV0370 (Mu50)
SA0357 (N315)
SAB0321 (RF122)

CGATAGATACAGCCAAAATACAATAAACGGAAAATCTAATAAATCTAGGA 250
CGATAGATACAGCCAAAATACAATAAACGGAAAATCTAATAAATCTAGGA 250
CGATAGATACAGCAAGGATACAATAAATGGAAAATCTAATAAATCTAGGA 250
CGATAGATACAGCAAGGATACAATAAATGGAAAATCTAATAAATCTAGGA 250
CGATAGATACAGCAAGGATACAATAAATGGAAAATCTAATAAATCTAGGA 250
CGATAGATACAGCAAGGATACAATAAATGGAAAATCTAATAAATCTAGGA 250
CGATAGATACAGCAAGGATACAATAAATGGAAAATCTAATAAATCTAGGA 250
CGATAGATACAGCAAGGATACAATAAATGGAAAATCTAATAAATCTAGGA 250
CGATAGATACAGCAAGGATACAATAAATGGAAAATCTAATAAATCTAGGA 250
CGATAGATACAGCAAGGATACAATAAATGGAAAATCTAATAAATCTAGGA 250
CGATAGATACAGCAAGGATACAATAAATGGAAAATCTAATAAATCTAGGA 250
CGATAGATACAGCCAAAATACAATAAACGGAAAATCTAATAAAGCTAGGA 250
***** * ***** *****

MW0345 (MW2)
SAS0347 (MSSA476)
SACOL0442 (COL)
SAOUHSC_00354 (NCTC8325)
NWMN_0362 (Newman)
SAUSA300_0370 (USA300-FPR3757)
SaurJH1_0429 (JH1)
SAHV_0367 (Mu3)
SaurJH9_0419 (JH9)
SAV0370 (Mu50)
SA0357 (N315)
SAB0321 (RF122)

ATTGGGTTTATTTCAGAGAGACCTTTAAATGAAAACCAAGTTCGTATAAAT 300
ATTGGGTTTATTTCAGAGAGACCTTTAAATGAAAACCAAGTTCGTATAAAT 300
ATTGGGTTTATTTCAGAGAGACCTTTAAATGAAAACCAAGTTCGTATACAT 300
ATTGGGTTTATTTCAGAGAGACCTTTAAATGAAAACCAAGTTCGTATACAT 300
ATTGGGTTTATTTCAGAGAGACCTTTAAATGAAAACCAAGTTCGTATACAT 300
ATTGGGTTTATTTCAGAGAGACCTTTAAATGAAAACCAAGTTCGTATACAT 300
ATTGGGTTTATTTCAGAGAGACCTTTAAATGAAAACCAAGTTCGTATACAT 300
ATTGGGTTTATTTCAGAGAGACCTTTAAATGAAAACCAAGTTCGTATACAT 300
ATTGGGTTTATTTCAGAGAGACCTTTAAATGAAAACCAAGTTCGTATACAT 300
ATTGGGTTTATTTCAGAGAGACCTTTAAATGAAAACCAAGTTCGTATACAT 300
ATTGGGTTTATTTCAGAGAGACCTTTAAATGAAAACCAAGTTCGTATACAT 300
ATTGGGTTTATTTCAGAGAGACCTTTAAATGAAAATCAAGTTCGTATACAT 300
***** *****

MW0345 (MW2)
SAS0347 (MSSA476)
SACOL0442 (COL)
SAOUHSC_00354 (NCTC8325)
NWMN_0362 (Newman)
SAUSA300_0370 (USA300-FPR3757)
SaurJH1_0429 (JH1)
SAHV_0367 (Mu3)
SaurJH9_0419 (JH9)
SAV0370 (Mu50)
SA0357 (N315)
SAB0321 (RF122)

TTAGAAGGAACATACAGAGTTGCTGATAGAGTATATACACCTAAGAGAAA 350
TTAGAAGGAACATACAGAGTTGCTGATAGAGTATATACACCTAAGAGAAA 350
TTAGAAGGAACATACACAGTTGCTGGCAGAGTGTATACACCTAAGAGGAA 350
TTAGAAGGAACATACACAGTTGCTGGCAGAGTGTATACACCTAAGAGGAA 350
TTAGAAGGAACATACACAGTTGCTGGCAGAGTGTATACACCTAAGAGGAA 350
TTAGAAGGAACATACACAGTTGCTGGCAGAGTGTATACACCTAAGAGGAA 350
TTAGAAGGAACATACACAGTTGCTGATAGAGTATATACACCTAAGAGAAA 350
TTAGAAGGAACATACACAGTTGCTGATAGAGTATATACACCTAAGAGAAA 350
TTAGAAGGAACATACACAGTTGCTGATAGAGTATATACACCTAAGAGAAA 350
TTAGAAGGAACATACACAGTTGCTGATAGAGTATATACACCTAAGAGAAA 350
TTAGAAGGAACATACACAGTTGCTGATAGAGTATATACACCTAAGAGAAA 350
TTAGAAGGATACATACAGAGTTGCTGATAGAGTGTATACACCTAAGAGGAA 350
***** *****

MW0345 (MW2)
SAS0347 (MSSA476)
SACOL0442 (COL)
SAOUHSC_00354 (NCTC8325)
NWMN_0362 (Newman)
SAUSA300_0370 (USA300-FPR3757)
SaurJH1_0429 (JH1)
SAHV_0367 (Mu3)
SaurJH9_0419 (JH9)
SAV0370 (Mu50)
SA0357 (N315)
SAB0321 (RF122)

TATTACTCTTAATAAAGAAGTTGTCACCTTTAAAGGAATTGGATCATATCA 400
TATTACTCTTAATAAAGAAGTTGTCACCTTTAAAGGAATTGGATCATATCA 400
TATTACTCTTAATAAAGAAGTTGTCACCTTTAAAGGAATTGGATCATATCA 400
TATTACTCTTAATAAAGAAGTTGTCACCTTTAAAGGAATTGGATCATATCA 400
TATTACTCTTAATAAAGAAGTTGTCACCTTTAAAGGAATTGGATCATATCA 400
TATTACTCTTAATAAAGAAGTTGTCACCTTTAAAGGAATTGGATCATATCA 400
TATTACTCTTAATAAAGAAGTTGTCACCTTTAAAGGAATTGGATCATATCA 400
TATTACTCTTAATAAAGAAGTTGTCACCTTTAAAGGAATTGGATCATATCA 400
TATTACTCTTAATAAAGAAGTTGTCACCTTTAAAGGAATTGGATCATATCA 400
TATTACTCTTAATAAAGAAGTTGTCACCTTTAAAGGAATTGGATCATATCA 400
TATTACTCTTAATAAAGAAGTTGTCACCTTTAAAGGAATTGGATCATATCA 400
TATTACTCTTAATAAAGAAGTTGTCACCTTTAAAGGAATTGGATCATATCA 400
CATTACTCTTAATAAAGAAGTTGTCACCTTTAAAGGAATTGGATCATATCA 400
*****

MW0345 (MW2)
SAS0347 (MSSA476)
SACOL0442 (COL)
SAOUHSC_00354 (NCTC8325)
NWMN_0362 (Newman)
SAUSA300_0370 (USA300-FPR3757)
SaurJH1_0429 (JH1)
SAHV_0367 (Mu3)
SaurJH9_0419 (JH9)
SAV0370 (Mu50)
SA0357 (N315)
SAB0321 (RF122)

TAAGATTTTGCTCATATTTCTTATGGCTTATATATGGGAGAACATTTGCCT 450
TAAGATTTTGCTCATATTTCTTATGGCTTATATATGGGAGAACATTTGCCT 450
TAAGATTTTGCTCATATTTCCCTATGGCTTGTATATGGGAGAACATTTGCCT 450
TAAGATTTTGCTCATATTTCCCTATGGCTTGTATATGGGAGAACATTTGCCT 450
TAAGATTTTGCTCATATTTCCCTATGGCTTGTATATGGGAGAACATTTGCCT 450
TAAGATTTTGCTCATATTTCCCTATGGCTTGTATATGGGAGAACATTTGCCT 450
TAAGATTTTGCTCATATTTCTTATGGCTTATATATGGGAGAACATTTGCCT 450
TAAGATTTTGCTCATATTTCTTATGGCTTATATATGGGAGAACATTTGCCT 450
TAAGATTTTGCTCATATTTCTTATGGCTTATATATGGGAGAACATTTGCCT 450
TAAGATTTTGCTCATATTTCTTATGGCTTATATATGGGAGAACATTTGCCT 450
TAAGATTTTGCTCATATTTCTTATGGCTTATATATGGGAGAACATTTGCCT 450
TAAGATTTTGCTCATATTTCTTATGGCTTATATATGGGAGAACATTTGCCT 450
TAAGATTTTGCTCATATTTCTTATGGCTTATATATGGGAGAACATTTGCCT 450
*****
```

FIG. 22B

\* \* \* \* \*

**II-SACOL0442 : Multiple polypeptide sequences alignment : selected epitopes shaded**

|                                |                                                        |     |
|--------------------------------|--------------------------------------------------------|-----|
| SACOL0442 (COL)                | MFKKYDSKNSIVLKSILSLGIIYGGTFGIYPKADASTQNSSSVQDKQLQK     | 50  |
| SAOUHSC_00354 (NCTC8325)       | MFKKYDSKNSIVLKSILSLGIIYGGTFGIYPKADASTQNSSSVQDKQLQK     | 50  |
| NWMN_0362 (Newman)             | MFKKYDSKNSIVLKSILSLGIIYGGTFGIYPKADASTQNSSSVQDKQLQK     | 50  |
| SAUSA300_0370 (USA300-FPR3757) | MFKKYDSKNSIVLKSILSLGIIYGGTFGIYPKADASTQNSSSVQDKQLQK     | 50  |
| SaurJH1_0429 (JH1)             | MFKKYDSKNSIVLKSILSLGIIYGGTFGIYPKADASTQNSSSVQDKQLQK     | 50  |
| SAHV_0367 (Mu3)                | MFKKYDSKNSIVLKSILSLGIIYGGTFGIYPKADASTQNSSSVQDKQLQK     | 50  |
| SaurJH9_0419 (JH9)             | MFKKYDSKNSIVLKSILSLGIIYGGTFGIYPKADASTQNSSSVQDKQLQK     | 50  |
| SAV0370 (Mu50)                 | MFKKYDSKNSIVLKSILSLGIIYGGTFGIYPKADASTQNSSSVQDKQLQK     | 50  |
| SA0357 (N315)                  | MFKKYDSKNSIVLKSILSLGIIYGGTFGIYPKADASTQNSSSVQDKQLQK     | 50  |
| SAS0347 (MSSA476)              | MFKKYDSKNSIVLKSILSLGIIYGGTFGIYPKADASTQNSSSVQDKQLQK     | 50  |
| SAB0321 (RF122)                | MFKKYDSKNSIVLKSILSLGIIYGGTFGIYPKADASTQNSSSVQDKQLQK     | 50  |
| Consensus                      | MFKKYDSKNSIVLKSILSLGIIYGGTFGIYPKADASTQNSSSVQDKQLQK     |     |
| SACOL0442 (COL)                | VEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIH     | 100 |
| SAOUHSC_00354 (NCTC8325)       | VEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIH     | 100 |
| NWMN_0362 (Newman)             | VEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIH     | 100 |
| SAUSA300_0370 (USA300-FPR3757) | VEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIH     | 100 |
| SaurJH1_0429 (JH1)             | VEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIH     | 100 |
| SAHV_0367 (Mu3)                | VEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIH     | 100 |
| SaurJH9_0419 (JH9)             | VEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIH     | 100 |
| SAV0370 (Mu50)                 | VEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIH     | 100 |
| SA0357 (N315)                  | VEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIH     | 100 |
| SAS0347 (MSSA476)              | VEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIH     | 100 |
| SAB0321 (RF122)                | VEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIH     | 100 |
| Consensus                      | VEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIH     |     |
| SACOL0442 (COL)                | LEGTYTVAGRVYTPKRNI TLNKEVVT LKELDHI IRFAHI SYGLYMGEHLP | 150 |
| SAOUHSC_00354 (NCTC8325)       | LEGTYTVAGRVYTPKRNI TLNKEVVT LKELDHI IRFAHI SYGLYMGEHLP | 150 |
| NWMN_0362 (Newman)             | LEGTYTVAGRVYTPKRNI TLNKEVVT LKELDHI IRFAHI SYGLYMGEHLP | 150 |
| SAUSA300_0370 (USA300-FPR3757) | LEGTYTVAGRVYTPKRNI TLNKEVVT LKELDHI IRFAHI SYGLYMGEHLP | 150 |
| SaurJH1_0429 (JH1)             | LEGTYTVAGRVYTPKRNI TLNKEVVT LKELDHI IRFAHI SYGLYMGEHLP | 150 |
| SAHV_0367 (Mu3)                | LEGTYTVAGRVYTPKRNI TLNKEVVT LKELDHI IRFAHI SYGLYMGEHLP | 150 |
| SaurJH9_0419 (JH9)             | LEGTYTVAGRVYTPKRNI TLNKEVVT LKELDHI IRFAHI SYGLYMGEHLP | 150 |
| SAV0370 (Mu50)                 | LEGTYTVAGRVYTPKRNI TLNKEVVT LKELDHI IRFAHI SYGLYMGEHLP | 150 |
| SA0357 (N315)                  | LEGTYTVAGRVYTPKRNI TLNKEVVT LKELDHI IRFAHI SYGLYMGEHLP | 150 |
| SAS0347 (MSSA476)              | LEGTYTVAGRVYTPKRNI TLNKEVVT LKELDHI IRFAHI SYGLYMGEHLP | 150 |
| SAB0321 (RF122)                | LEGTYTVAGRVYTPKRNI TLNKEVVT LKELDHI IRFAHI SYGLYMGEHLP | 150 |
| Consensus                      | LEGTYTVAGRVYTPKRNI TLNKEVVT LKELDHI IRFAHI SYGLYMGEHLP |     |
| SACOL0442 (COL)                | KGNIVINTKGGKYTLESHKELQKDRENVKINTADIKNVTFLVKVSVNDI      | 200 |
| SAOUHSC_00354 (NCTC8325)       | KGNIVINTKGGKYTLESHKELQKDRENVKINTADIKNVTFLVKVSVNDI      | 200 |
| NWMN_0362 (Newman)             | KGNIVINTKGGKYTLESHKELQKDRENVKINTADIKNVTFLVKVSVNDI      | 200 |
| SAUSA300_0370 (USA300-FPR3757) | KGNIVINTKGGKYTLESHKELQKDRENVKINTADIKNVTFLVKVSVNDI      | 200 |
| SaurJH1_0429 (JH1)             | KGNIVINTKGGKYTLESHKELQKDRENVKINTADIKNVTFLVKVSVNDI      | 200 |
| SAHV_0367 (Mu3)                | KGNIVINTKGGKYTLESHKELQKDRENVKINTADIKNVTFLVKVSVNDI      | 200 |
| SaurJH9_0419 (JH9)             | KGNIVINTKGGKYTLESHKELQKDRENVKINTADIKNVTFLVKVSVNDI      | 200 |
| SAV0370 (Mu50)                 | KGNIVINTKGGKYTLESHKELQKDRENVKINTADIKNVTFLVKVSVNDI      | 200 |
| SA0357 (N315)                  | KGNIVINTKGGKYTLESHKELQKDRENVKINTADIKNVTFLVKVSVNDI      | 200 |
| SAS0347 (MSSA476)              | KGNIVINTKGGKYTLESHKELQKDRENVKINTADIKNVTFLVKVSVNDI      | 200 |
| SAB0321 (RF122)                | KGNIVINTKGGKYTLESHKELQKDRENVKINTADIKNVTFLVKVSVNDI      | 200 |
| Consensus                      | KGNIVINTKGGKYTLESHKELQKDRENVKINTADIKNVTFLVKVSVNDI      |     |
| SACOL0442 (COL)                | EQV                                                    | 203 |
| SAOUHSC_00354 (NCTC8325)       | EQV                                                    | 203 |
| NWMN_0362 (Newman)             | EQV                                                    | 203 |
| SAUSA300_0370 (USA300-FPR3757) | EQV                                                    | 203 |
| SaurJH1_0429 (JH1)             | EQV                                                    | 203 |
| SAHV_0367 (Mu3)                | EQV                                                    | 203 |
| SaurJH9_0419 (JH9)             | EQV                                                    | 203 |
| SAV0370 (Mu50)                 | EQV                                                    | 203 |
| SA0357 (N315)                  | EQV                                                    | 203 |
| SAS0347 (MSSA476)              | EQV                                                    | 203 |
| SAB0321 (RF122)                | EQV                                                    | 203 |
| Consensus                      | EQV                                                    | 203 |

**FIG. 22D**

**I- SACOL0720 : Multiple polynucleotide sequences alignment**

```

SaurJH1_0700 (JH1) -----
SaurJH9_0685 (JH9) -----
SAHV_0659 (Mu3) -----
SAV0662 (Mu50) ATGACCTTTAACGAGATAATATTTAAAAATTTCCGTCAAAATTTATCACA 50
SA0617 (N315) ATGACCTTTAACGAGATAATATTTAAAAATTTCCGTCAAAATTTATCACA 50
MW0624 (MW2) ATGACCTTTAACGAGATAATATTTAAAAATTTCCGTCAAAATTTATCACA 50
SAS0627 (MSSA476) ATGACCTTTAACGAGATAATATTTAAAAATTTCCGTCAAAATTTATCACA 50
SACOL0720 ATGACCTTTAACGAGATAATATTTAAAAATTTCCGTCAAAATTTATCACA 50
SAUSA300_0648 (USA300-FPR3757) ATGACCTTTAACGAGATAATATTTAAAAATTTCCGTCAAAATTTATCACA 50
SAOUHSC_00668 (NCTC8325) -----
NWMN_0631 (Newman) -----
SAB0611 (RF122) ATGACCTTTAACGAGATAATATTTAAAAATTTCCGTCAAAATTTATCACA 50

SaurJH1_0700 (JH1) -----
SaurJH9_0685 (JH9) -----
SAHV_0659 (Mu3) -----
SAV0662 (Mu50) TTATGCCATCTATCTTTTTTTCATTAATTACGAGTGTAGTATTGTATTTTA 100
SA0617 (N315) TTATGCCATCTATCTTTTTTTCATTAATTACGAGTGTAGTATTGTATTTTA 100
MW0624 (MW2) TTATGCCATCTATCTTTTTTTCATTAATTACGAGTGTAGTATTGTATTTTA 100
SAS0627 (MSSA476) TTATGCCATCTATCTTTTTTTCATTAATTACGAGTGTAGTATTGTATTTTA 100
SACOL0720 TTATGCCATCTATCTTTTTTTCGTTAATTACGAGTGTAGTATTGTATTTTA 100
SAUSA300_0648 (USA300-FPR3757) TTATGCCATCTATCTTTTTTTCGTTAATTACGAGTGTAGTATTGTATTTTA 100
SAOUHSC_00668 (NCTC8325) -----TTGTATTTTA 10
NWMN_0631 (Newman) -----
SAB0611 (RF122) TTATGCCATCTATCTTTTTTTCATTAATTACGAGTGTAGTATTGTATTTTA 100

SaurJH1_0700 (JH1) -----ATGACAGAGTCATAT 15
SaurJH9_0685 (JH9) -----ATGACAGAGTCATAT 15
SAHV_0659 (Mu3) -----ATGACAGAGTCATAT 15
SAV0662 (Mu50) GCTTTGTAGCATTAAAAATACGCGCATAACTAAACATGACAGAGTCATAT 150
SA0617 (N315) GCTTTGTAGCATTAAAAATACGCGCATAACTAAACATGACAGAGTCATAT 150
MW0624 (MW2) GCTTTGTAGCATTAAAAATACGCGCATAACTAAACATGACAGAGTCATAT 150
SAS0627 (MSSA476) GCTTTGTAGCATTAAAAATACGCGCATAACTAAACATGACAGAGTCATAT 150
SACOL0720 GCTTTGTAGCATTAAAAATACGCTCATAAACTAAACATGACAGAGTCATAT 150
SAUSA300_0648 (USA300-FPR3757) GCTTTGTAGCATTAAAAATACGCTCATAAACTAAACATGACAGAGTCATAT 150
SAOUHSC_00668 (NCTC8325) GCTTTGTAGCATTAAAAATACGCTCATAAACTAAACATGACAGAGTCATAT 60
NWMN_0631 (Newman) -----ATGACAGAGTCATAT 15
SAB0611 (RF122) GCTTTGTAGCATTAAAAATACGCTCATAAACTAAACATGACAGAGTCATAT 150
*****

SaurJH1_0700 (JH1) CCAATTATTAAGGAAGGCTCACAAGTCGGAAGCTACTTTCTATTTTTTCAT 65
SaurJH9_0685 (JH9) CCAATTATTAAGGAAGGCTCACAAGTCGGAAGCTACTTTCTATTTTTTCAT 65
SAHV_0659 (Mu3) CCAATTATTAAGGAAGGCTCACAAGTCGGAAGCTACTTTCTATTTTTTCAT 65
SAV0662 (Mu50) CCAATTATTAAGGAAGGCTCACAAGTCGGAAGCTACTTTCTATTTTTTCAT 200
SA0617 (N315) CCAATTATTAAGGAAGGCTCACAAGTCGGAAGCTACTTTCTATTTTTTCAT 200
MW0624 (MW2) CCAATTATTAAGGAAGGCTCACAAGTCGGAAGCTACTTTCTATTTTTTCAT 200
SAS0627 (MSSA476) CCAATTATTAAGGAAGGCTCACAAGTCGGAAGCTACTTTCTATTTTTTCAT 200
SACOL0720 CCAATTATTAAGGAAGGCTCACAAGTCGGAAGCTACTTTCTATTTTTTCAT 200
SAUSA300_0648 (USA300-FPR3757) CCAATTATTAAGGAAGGCTCACAAGTCGGAAGCTACTTTCTATTTTTTCAT 200
SAOUHSC_00668 (NCTC8325) CCAATTATTAAGGAAGGCTCACAAGTCGGAAGCTACTTTCTATTTTTTCAT 110
NWMN_0631 (Newman) CCAATTATTAAGGAAGGCTCACAAGTCGGAAGCTACTTTCTATTTTTTCAT 65
SAB0611 (RF122) CCAATTATTAAGGAAGGCTCACAAGTCGGAAGCTACTTTCTATTTTTTCAT 200
*****

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**FIG. 23A**

|                                |                                                     |     |
|--------------------------------|-----------------------------------------------------|-----|
| SaurJH1_0700 (JH1)             | CATAATTGCATTTTTGTTATATGCCAATGTGTTATTTATTAAACGACGAA  | 115 |
| SaurJH9_0685 (JH9)             | CATAATTGCATTTTTGTTATATGCCAATGTGTTATTTATTAAACGACGAA  | 115 |
| SAHV_0659 (Mu3)                | CATAATTGCATTTTTGTTATATGCCAATGTGTTATTTATTAAACGACGAA  | 115 |
| SAV0662 (Mu50)                 | CATAATTGCATTTTTGTTATATGCCAATGTGTTATTTATTAAACGACGAA  | 250 |
| SA0617 (N315)                  | CATAATTGCATTTTTGTTATATGCCAATGTGTTATTTATTAAACGACGAA  | 250 |
| MW0624 (MW2)                   | CATAATTGCATTTTTGTTATATGCCAATGTGTTATTTATTAAACGACGAA  | 250 |
| SAS0627 (MSSA476)              | CATAATTGCATTTTTGTTATATGCCAATGTGTTATTTATTAAACGACGAA  | 250 |
| SACOL0720_                     | CATAATTGCATTTTTGTTATATGCCAATGTGTTATTTATTAAACGACGAA  | 250 |
| SAUSA300_0648 (USA300-FPR3757) | CATAATTGCATTTTTGTTATATGCCAATGTGTTATTTATTAAACGACGAA  | 250 |
| SAOUHSC_00668 (NCTC8325)       | CATAATTGCATTTTTGTTATATGCCAATGTGTTATTTATTAAACGACGAA  | 160 |
| NWMN_0631 (Newman)             | CATAATTGCATTTTTGTTATATGCCAATGTGTTATTTATTAAACGACGAA  | 115 |
| SAB0611 (RF122)                | CATAATTGCATTTTTGTTATATGCCAATGTGTTATTTATTAAACGACGAA  | 250 |
| *****                          |                                                     |     |
| SaurJH1_0700 (JH1)             | GTTATGAGCTTGCATTATATCAAACATTAGGTTTATCTAAATTCAACATT  | 165 |
| SaurJH9_0685 (JH9)             | GTTATGAGCTTGCATTATATCAAACATTAGGTTTATCTAAATTCAACATT  | 165 |
| SAHV_0659 (Mu3)                | GTTATGAGCTTGCATTATATCAAACATTAGGTTTATCTAAATTCAACATT  | 165 |
| SAV0662 (Mu50)                 | GTTATGAGCTTGCATTATATCAAACATTAGGTTTATCTAAATTCAACATT  | 300 |
| SA0617 (N315)                  | GTTATGAGCTTGCATTATATCAAACATTAGGTTTATCTAAATTCAACATT  | 300 |
| MW0624 (MW2)                   | GTTATGAGCTTGCATTATATCAAACATTAGGTTTATCTAAATTCAACATT  | 300 |
| SAS0627 (MSSA476)              | GTTATGAGCTTGCATTATATCAAACATTAGGTTTATCTAAATTCAACATT  | 300 |
| SACOL0720_                     | GTTATGAGCTTGCATTATATCAAACATTAGGTTTATCTAAATTCAACATT  | 300 |
| SAUSA300_0648 (USA300-FPR3757) | GTTATGAGCTTGCATTATATCAAACATTAGGTTTATCTAAATTCAACATT  | 300 |
| SAOUHSC_00668 (NCTC8325)       | GTTATGAGCTTGCATTATATCAAACATTAGGTTTATCTAAATTCAACATT  | 210 |
| NWMN_0631 (Newman)             | GTTATGAGCTTGCATTATATCAAACATTAGGTTTATCTAAATTCAACATT  | 165 |
| SAB0611 (RF122)                | GTTATGAGCTTGCATTATATCAAACATTAGGTTTATCTAAATTCAACATT  | 300 |
| *****                          |                                                     |     |
| SaurJH1_0700 (JH1)             | ATTTATATACTAATGCTCGAACAATTACTAATATTTATAAATTACGGCAAT | 215 |
| SaurJH9_0685 (JH9)             | ATTTATATACTAATGCTCGAACAATTACTAATATTTATAAATTACGGCAAT | 215 |
| SAHV_0659 (Mu3)                | ATTTATATACTAATGCTCGAACAATTACTAATATTTATAAATTACGGCAAT | 215 |
| SAV0662 (Mu50)                 | ATTTATATACTAATGCTCGAACAATTACTAATATTTATAAATTACGGCAAT | 350 |
| SA0617 (N315)                  | ATTTATATACTAATGCTCGAACAATTACTAATATTTATAAATTACGGCAAT | 350 |
| MW0624 (MW2)                   | ATTTATATACTAATGCTCGAACAATTACTAATATTTATAAATTACGGCAAT | 350 |
| SAS0627 (MSSA476)              | ATTTATATACTAATGCTCGAACAATTACTAATATTTATAAATTACGGCAAT | 350 |
| SACOL0720_                     | ATTTATATACTAATGCTCGAACAATTACTAATATTTATAAATTACGGCAAT | 350 |
| SAUSA300_0648 (USA300-FPR3757) | ATTTATATACTAATGCTCGAACAATTACTAATATTTATAAATTACGGCAAT | 350 |
| SAOUHSC_00668 (NCTC8325)       | ATTTATATACTAATGCTCGAACAATTACTAATATTTATAAATTACGGCAAT | 260 |
| NWMN_0631 (Newman)             | ATTTATATACTAATGCTCGAACAATTACTAATATTTATAAATTACGGCAAT | 215 |
| SAB0611 (RF122)                | ATTTATATACTAATGCTCGAACAATTACTAATATTTATAAATTACGGCAAT | 350 |
| *****                          |                                                     |     |
| SaurJH1_0700 (JH1)             | ATTAGGTATTATTATTGGTATTTTTGGTTCAAACCTGTTATTAATGATTG  | 265 |
| SaurJH9_0685 (JH9)             | ATTAGGTATTATTATTGGTATTTTTGGTTCAAACCTGTTATTAATGATTG  | 265 |
| SAHV_0659 (Mu3)                | ATTAGGTATTATTATTGGTATTTTTGGTTCAAACCTGTTATTAATGATTG  | 265 |
| SAV0662 (Mu50)                 | ATTAGGTATTATTATTGGTATTTTTGGTTCAAACCTGTTATTAATGATTG  | 400 |
| SA0617 (N315)                  | ATTAGGTATTATTATTGGTATTTTTGGTTCAAACCTGTTATTAATGATTG  | 400 |
| MW0624 (MW2)                   | ATTAGGTATTATTATTGGTATTTTTGGTTCAAACCTGTTATTAATGATTG  | 400 |
| SAS0627 (MSSA476)              | ATTAGGTATTATTATTGGTATTTTTGGTTCAAACCTGTTATTAATGATTG  | 400 |
| SACOL0720_                     | ATTAGGTATTATTATTGGTATTTTTGGTTCAAACCTGTTATTAATGATTG  | 400 |
| SAUSA300_0648 (USA300-FPR3757) | ATTAGGTATTATTATTGGTATTTTTGGTTCAAACCTGTTATTAATGATTG  | 400 |
| SAOUHSC_00668 (NCTC8325)       | ATTAGGTATTATTATTGGTATTTTTGGTTCAAACCTGTTATTAATGATTG  | 310 |
| NWMN_0631 (Newman)             | ATTAGGTATTATTATTGGTATTTTTGGTTCAAACCTGTTATTAATGATTG  | 265 |
| SAB0611 (RF122)                | ATTAGGTATTATTATTGGTATTTTTGGTTCAAACCTGTTATTAATGATTG  | 400 |
| *****                          |                                                     |     |
| SaurJH1_0700 (JH1)             | TCCTTACATTATTAGGAATTAAGAAAAGGTTCCAATTATTTTTAGTTTG   | 315 |
| SaurJH9_0685 (JH9)             | TCCTTACATTATTAGGAATTAAGAAAAGGTTCCAATTATTTTTAGTTTG   | 315 |
| SAHV_0659 (Mu3)                | TCCTTACATTATTAGGAATTAAGAAAAGGTTCCAATTATTTTTAGTTTG   | 315 |
| SAV0662 (Mu50)                 | TCCTTACATTATTAGGAATTAAGAAAAGGTTCCAATTATTTTTAGTTTG   | 450 |
| SA0617 (N315)                  | TCCTTACATTATTAGGAATTAAGAAAAGGTTCCAATTATTTTTAGTTTG   | 450 |
| MW0624 (MW2)                   | TCCTTACATTATTAGGAATTAAGAAAAGGTTCCAATTATTTTTAGTTTG   | 450 |
| SAS0627 (MSSA476)              | TCCTTACATTATTAGGAATTAAGAAAAGGTTCCAATTATTTTTAGTTTG   | 450 |
| SACOL0720_                     | TCCTTACATTATTAGGAATTAAGAAAAGGTTCCAATTATTTTTAGTTTG   | 450 |
| SAUSA300_0648 (USA300-FPR3757) | TCCTTACATTATTAGGAATTAAGAAAAGGTTCCAATTATTTTTAGTTTG   | 450 |
| SAOUHSC_00668 (NCTC8325)       | TCCTTACATTATTAGGAATTAAGAAAAGGTTCCAATTATTTTTAGTTTG   | 360 |
| NWMN_0631 (Newman)             | TCCTTACATTATTAGGAATTAAGAAAAGGTTCCAATTATTTTTAGTTTG   | 315 |
| SAB0611 (RF122)                | TCCTTACATTATTAGGAATTAAGAAAAGGTTCCAATTATTTTTAGTTTG   | 450 |
| *****                          |                                                     |     |

FIG. 23B

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SaurJH1_0700 (JH1)      AGGGCGGTATTTTGAACATTAATGTTAATCGGTGTCGCTTATTTTTTAAC 365
SaurJH9_0685 (JH9)      AGGGCGGTATTTTGAACATTAATGTTAATCGGTGTCGCTTATTTTTTAAC 365
SAHV_0659 (Mu3)         AGGGCGGTATTTTGAACATTAATGTTAATCGGTGTCGCTTATTTTTTAAC 365
SAV0662 (Mu50)          AGGGCGGTATTTTGAACATTAATGTTAATCGGTGTCGCTTATTTTTTAAC 500
SA0617 (N315)           AGGGCGGTATTTTGAACATTAATGTTAATCGGTGTCGCTTATTTTTTAAC 500
MW0624 (MW2)            AGGGCGGTATTTTGAACATTAATGTTAATCGGTGTCGCTTATTTTTTAAC 500
SAS0627 (MSSA476)       AGGGCGGTATTTTGAACATTAATGTTAATCGGTGTCGCTTATTTTTTAAC 500
SACOL0720_              AGGGCGGTATTTTGAACATTAATGTTAATCGGTGTCGCTTATTTTTTAAC 500
SAUSA300_0648 (USA300-FPR3757) AGGGCGGTATTTTGAACATTAATGTTAATCGGTGTCGCTTATTTTTTAAC 500
SAOUHSC_00668 (NCTC8325) AGGGCGGTATTTTGAACATTAATGTTAATCGGTGTCGCTTATTTTTTAAC 410
NWMN_0631 (Newman)      AGGGCGGTATTTTGAACATTAATGTTAATCGGTGTCGCTTATTTTTTAAC 365
SAB0611 (RF122)         AGGGCGGTATTTTGAACATTAATGTTAATCGGTGTCGCTTATTTTTTAAC 500
*****

SaurJH1_0700 (JH1)      CTCTGCTCAAAATTTTATATTAGTGTTCAAACAATCTATTTTCACAGATGT 415
SaurJH9_0685 (JH9)      CTCTGCTCAAAATTTTATATTAGTGTTCAAACAATCTATTTTCACAGATGT 415
SAHV_0659 (Mu3)         CTCTGCTCAAAATTTTATATTAGTGTTCAAACAATCTATTTTCACAGATGT 415
SAV0662 (Mu50)          CTCTGCTCAAAATTTTATATTAGTGTTCAAACAATCTATTTTCACAGATGT 550
SA0617 (N315)           CTCTGCTCAAAATTTTATATTAGTGTTCAAACAATCTATTTTCACAGATGT 550
MW0624 (MW2)            CTCTGCTCAAAATTTTATATTAGTGTTCAAACAATCTATTTTCACAGATGT 550
SAS0627 (MSSA476)       CTCTGCTCAAAATTTTATATTAGTGTTCAAACAATCTATTTTCACAGATGT 550
SACOL0720_              ATCTGCTCAAAATTTTATATTAGTGTTCAAACAATCTATTTTCACAGATGT 550
SAUSA300_0648 (USA300-FPR3757) ATCTGCTCAAAATTTTATATTAGTGTTCAAACAATCTATTTTCACAGATGT 550
SAOUHSC_00668 (NCTC8325) ATCTGCTCAAAATTTTATATTAGTGTTCAAACAATCTATTTTCACAGATGT 460
NWMN_0631 (Newman)      ATCTGCTCAAAATTTTATATTAGTGTTCAAACAATCTATTTTCACAGATGT 415
SAB0611 (RF122)         ATCTGCTCAAAATTTTATATTAGTGTTCAAACAATCTATTTTCACAGATGT 550
*****

SaurJH1_0700 (JH1)      CAAAGAATAACCCAGGTAAAGAAACAAATCATAATAAAATTACATTTGAA 465
SaurJH9_0685 (JH9)      CAAAGAATAACCCAGGTAAAGAAACAAATCATAATAAAATTACATTTGAA 465
SAHV_0659 (Mu3)         CAAAGAATAACCCAGGTAAAGAAACAAATCATAATAAAATTACATTTGAA 465
SAV0662 (Mu50)          CAAAGAATAACCCAGGTAAAGAAACAAATCATAATAAAATTACATTTGAA 600
SA0617 (N315)           CAAAGAATAACCCAGGTAAAGAAACAAATCATAATAAAATTACATTTGAA 600
MW0624 (MW2)            CAAAGAATAACCCAGGTAAAGAAACAAATCATAATAAAATTACATTTGAA 600
SAS0627 (MSSA476)       CAAAGAATAACCCAGGTAAAGAAACAAATCATAATAAAATTACATTTGAA 600
SACOL0720_              CAAAGAATAACCCAGGTAAAGAAACAAATCATAATAAAATTACATTTGAA 600
SAUSA300_0648 (USA300-FPR3757) CAAAGAATAACCCAGGTAAAGAAACAAATCATAATAAAATTACATTTGAA 600
SAOUHSC_00668 (NCTC8325) CAAAGAATAACCCAGGTAAAGAAACAAATCATAATAAAATTACATTTGAA 510
NWMN_0631 (Newman)      CAAAGAATAACCCAGGTAAAGAAACAAATCATAATAAAATTACATTTGAA 465
SAB0611 (RF122)         CAAAGAATAACCCAGGTAAAGAAACAAATCATAATAAAATTACATTTGAA 600
*****

SaurJH1_0700 (JH1)      GAGGTGTGTTTTAGGCATCTTAGGTATAGTATTGATTATCACAGGATACTA 515
SaurJH9_0685 (JH9)      GAGGTGTGTTTTAGGCATCTTAGGTATAGTATTGATTATCACAGGATACTA 515
SAHV_0659 (Mu3)         GAGGTGTGTTTTAGGCATCTTAGGTATAGTATTGATTACCACAGGATACTA 515
SAV0662 (Mu50)          GAGGTGTGTTTTAGGCATCTTAGGTATAGTATTGATTACCACAGGATACTA 650
SA0617 (N315)           GAGGTGTGTTTTAGGCATCTTAGGTATAGTATTGATTACCACAGGATACTA 650
MW0624 (MW2)            GAGGTGTGTTTTAGGCATCTTAGGTATAGTATTGATTACCACAGGATACTA 650
SAS0627 (MSSA476)       GAGGTGTGTTTTAGGCATCTTAGGTATAGTATTGATTACCACAGGATACTA 650
SACOL0720_              GAGGTGTGTTTTAGGCATCTTAGGTATAGTATTGATTACCACAGGATACTA 650
SAUSA300_0648 (USA300-FPR3757) GAGGTGTGTTTTAGGCATCTTAGGTATAGTATTGATTACCACAGGATACTA 650
SAOUHSC_00668 (NCTC8325) GAGGTGTGTTTTAGGCATCTTAGGTATAGTATTGATTACCACAGGATACTA 560
NWMN_0631 (Newman)      GAGGTGTGTTTTAGGCATCTTAGGTATAGTATTGATTACCACAGGATACTA 515
SAB0611 (RF122)         GAGGTGTGTTTTAGGCATCTTAGGTATAGTATTGATTACCACAGGATACTA 650
*****

SaurJH1_0700 (JH1)      TCTATCTTTGAACATTGTTCAATATTATGATTCTATCGGTATACTTATGT 565
SaurJH9_0685 (JH9)      TCTATCTTTGAACATTGTTCAATATTATGATTCTATCGGTATACTTATGT 565
SAHV_0659 (Mu3)         TCTATCTTTGAACATTGTTCAATATTATGATTCTATCGGTATACTTATGT 565
SAV0662 (Mu50)          TCTATCTTTGAACATTGTTCAATATTATGATTCTATCGGTATACTTATGT 700
SA0617 (N315)           TCTATCTTTGAACATTGTTCAATATTATGATTCTATCGGTATACTTATGT 700
MW0624 (MW2)            TCTATCTTTGAACATTGTTCAATATTATGATTCTATCGGTATACTTATGT 700
SAS0627 (MSSA476)       TCTATCTTTGAACATTGTTCAATATTATGATTCTATCGGTATACTTATGT 700
SACOL0720_              TCTATCTTTGAACATTGTTCAATATTATGATTCTATCGGTATACTTATGT 700
SAUSA300_0648 (USA300-FPR3757) TCTATCTTTGAACATTGTTCAATATTATGATTCTATCGGTATACTTATGT 700
SAOUHSC_00668 (NCTC8325) TCTATCTTTGAACATTGTTCAATATTATGATTCTATCGGTATACTTATGT 700
NWMN_0631 (Newman)      TCTATCTTTGAACATTGTTCAATATTATGATTCTATCGGTATACTTATGT 610
SAB0611 (RF122)         TCTATCTTTGAACATTGTTCAATATTATGATTCTATCGGTATACTTATGT 700
*****

```

FIG. 23C

|                                |                                                     |     |
|--------------------------------|-----------------------------------------------------|-----|
| SaurJH1_0700 (JH1)             | TTATTTTATTGTCAACTGTGATTGGGGCATACTTATTTTAAAAAGCTCT   | 615 |
| SaurJH9_0685 (JH9)             | TTATTTTATTGTCAACTGTGATTGGGGCATACTTATTTTAAAAAGCTCT   | 615 |
| SAHV_0659 (Mu3)                | TTATTTTATTGTCAACTGTGATTGGGGCATACTTATTTTAAAAAGCTCT   | 615 |
| SAV0662 (Mu50)                 | TTATTTTATTGTCAACTGTGATTGGGGCATACTTATTTTAAAAAGCTCT   | 750 |
| SA0617 (N315)                  | TTATTTTATTGTCAACTGTGATTGGGGCATACTTATTTTAAAAAGCTCT   | 750 |
| MW0624 (MW2)                   | TTATTTTATTGTCAACTGTGATTGGGGCATACTTATTTTAAAAAGCTCT   | 750 |
| SAS0627 (MSSA476)              | TTATTTTATTGTCAACTGTGATTGGGGCATACTTATTTTAAAAAGCTCT   | 750 |
| SACOL0720_                     | TTATTTTATTGTCAACTGTGATTGGGGCATACTTATTTTAAAAAGCTCT   | 750 |
| SAUSA300_0648 (USA300-FPR3757) | TTATTTTATTGTCAACTGTGATTGGGGCATACTTATTTTAAAAAGCTCT   | 750 |
| SAOUHSC_00668 (NCTC8325)       | TTATTTTATTGTCAACTGTGATTGGGGCATACTTATTTTAAAAAGCTCT   | 660 |
| NWMN_0631 (Newman)             | TTATTTTATTGTCAACTGTGATTGGGGCATACTTATTTTAAAAAGCTCT   | 615 |
| SAB0611 (RF122)                | TTATTTTATTGTCAACTGTGATTGGGGCATACTTATTTTAAAAAGCTCT   | 750 |
|                                | *****                                               |     |
| SaurJH1_0700 (JH1)             | GTTTCTCTAGTTTTTAAAAATGGTGAAGAAGTTTAGAAAAGGTGTTATAAG | 665 |
| SaurJH9_0685 (JH9)             | GTTTCTCTAGTTTTTAAAAATGGTGAAGAAGTTTAGAAAAGGTGTTATAAG | 665 |
| SAHV_0659 (Mu3)                | GTTTCTCTAGTTTTTAAAAATGGTGAAGAAGTTTAGAAAAGGTGTTATAAG | 665 |
| SAV0662 (Mu50)                 | GTTTCTCTAGTTTTTAAAAATGGTGAAGAAGTTTAGAAAAGGTGTTATAAG | 800 |
| SA0617 (N315)                  | GTTTCTCTAGTTTTTAAAAATGGTGAAGAAGTTTAGAAAAGGTGTTATAAG | 800 |
| MW0624 (MW2)                   | GTTTCTCTAGTTTTTAAAAATGGTGAAGAAGTTTAGAAAAGGTGTTATAAG | 800 |
| SAS0627 (MSSA476)              | GTTTCTCTAGTTTTTAAAAATGGTGAAGAAGTTTAGAAAAGGTGTTATAAG | 800 |
| SACOL0720_                     | GTTTCTCTAGTTTTTAAAAATGGTGAAGAAGTTTAGAAAAGGTGTTATAAG | 800 |
| SAUSA300_0648 (USA300-FPR3757) | GTTTCTCTAGTTTTTAAAAATGGTGAAGAAGTTTAGAAAAGGTGTTATAAG | 800 |
| SAOUHSC_00668 (NCTC8325)       | GTTTCTCTAGTTTTTAAAAATGGTGAAGAAGTTTAGAAAAGGTGTTATAAG | 710 |
| NWMN_0631 (Newman)             | GTTTCTCTAGTTTTTAAAAATGGTGAAGAAGTTTAGAAAAGGTGTTATAAG | 665 |
| SAB0611 (RF122)                | GTTTCTCTAGTTTTTAAAAATGGTGAAGAAGTTTAGAAAAGGTGTTATAAG | 800 |
|                                | *****                                               |     |
| SaurJH1_0700 (JH1)             | TGTAATGATGTCATGTTCTCATCATCTATTATGTATCGTATTAAGAAAA   | 715 |
| SaurJH9_0685 (JH9)             | TGTAATGATGTCATGTTCTCATCATCTATTATGTATCGTATTAAGAAAA   | 715 |
| SAHV_0659 (Mu3)                | TGTAATGATGTCATGTTCTCATCATCTATTATGTATCGTATTAAGAAAA   | 715 |
| SAV0662 (Mu50)                 | TGTAATGATGTCATGTTCTCATCATCTATTATGTATCGTATTAAGAAAA   | 850 |
| SA0617 (N315)                  | TGTAATGATGTCATGTTCTCATCATCTATTATGTATCGTATTAAGAAAA   | 850 |
| MW0624 (MW2)                   | TGTAATGATGTCATGTTCTCATCATCTATTATGTATCGTATTAAGAAAA   | 850 |
| SAS0627 (MSSA476)              | TGTAATGATGTCATGTTCTCATCATCTATTATGTATCGTATTAAGAAAA   | 850 |
| SACOL0720_                     | TGTAATGATGTCATGTTCTCATCATCTATTATGTATCGTATTAAGAAAA   | 850 |
| SAUSA300_0648 (USA300-FPR3757) | TGTAATGATGTCATGTTCTCATCATCTATTATGTATCGTATTAAGAAAA   | 850 |
| SAOUHSC_00668 (NCTC8325)       | TGTAATGATGTCATGTTCTCATCATCTATTATGTATCGTATTAAGAAAA   | 760 |
| NWMN_0631 (Newman)             | TGTAATGATGTCATGTTCTCATCATCTATTATGTATCGTATTAAGAAAA   | 715 |
| SAB0611 (RF122)                | TGTAATGATGTCATGTTCTCATCATCTATTATGTATCGTATTAAGAAAA   | 850 |
|                                | *****                                               |     |
| SaurJH1_0700 (JH1)             | ATGCTTTTTCACTTACGGTCATGGCAATCATTTTCAGCGATTACTGTTTCA | 765 |
| SaurJH9_0685 (JH9)             | ATGCTTTTTCACTTACGGTCATGGCAATCATTTTCAGCGATTACTGTTTCA | 765 |
| SAHV_0659 (Mu3)                | ATGCTTTTTCACTTACGGTCATGGCAATCATTTTCAGCGATTACTGTTTCA | 765 |
| SAV0662 (Mu50)                 | ATGCTTTTTCACTTACGGTCATGGCAATCATTTTCAGCGATTACTGTTTCA | 900 |
| SA0617 (N315)                  | ATGCTTTTTCACTTACGGTCATGGCAATCATTTTCAGCGATTACTGTTTCA | 900 |
| MW0624 (MW2)                   | ATGCTTTTTCACTTACGGTCATGGCAATCATTTTCAGCGATTACTGTTTCA | 900 |
| SAS0627 (MSSA476)              | ATGCTTTTTCACTTACGGTCATGGCAATCATTTTCAGCGATTACTGTTTCA | 900 |
| SACOL0720_                     | ATGCTTTTTCACTTACGGTCATGGCAATCATTTTCAGCGATTACTGTTTCA | 900 |
| SAUSA300_0648 (USA300-FPR3757) | ATGCTTTTTCACTTACGGTCATGGCAATCATTTTCAGCGATTACTGTTTCA | 900 |
| SAOUHSC_00668 (NCTC8325)       | ATGCTTTTTCACTTACGGTCATGGCAATCATTTTCAGCGATTACTGTTTCA | 810 |
| NWMN_0631 (Newman)             | ATGCTTTTTCACTTACGGTCATGGCAATCATTTTCAGCGATTACTGTTTCA | 765 |
| SAB0611 (RF122)                | ATGCTTTTTCACTTACGGTCATGGCAATCATTTTCAGCGATTACTGTTTCA | 900 |
|                                | *****                                               |     |
| SaurJH1_0700 (JH1)             | GTTCTTTTGCTTTGCTGCTATAAGTAGAGCGTCCTTATCAAGTGAAATAAA | 815 |
| SaurJH9_0685 (JH9)             | GTTCTTTTGCTTTGCTGCTATAAGTAGAGCGTCCTTATCAAGTGAAATAAA | 815 |
| SAHV_0659 (Mu3)                | GTTCTTTTGCTTTGCTGCTATAAGTAGAGCGTCCTTATCAAGTGAAATAAA | 815 |
| SAV0662 (Mu50)                 | GTTCTTTTGCTTTGCTGCTATAAGTAGAGCGTCCTTATCAAGTGAAATAAA | 950 |
| SA0617 (N315)                  | GTTCTTTTGCTTTGCTGCTATAAGTAGAGCGTCCTTATCAAGTGAAATAAA | 950 |
| MW0624 (MW2)                   | GTTCTTTTGCTTTGCTGCTATAAGTAGAGCGTCCTTATCAAGTGAAATAAA | 950 |
| SAS0627 (MSSA476)              | GTTCTTTTGCTTTGCTGCTATAAGTAGAGCGTCCTTATCAAGTGAAATAAA | 950 |
| SACOL0720_                     | GTTCTTTTGCTTTGCTGCTATAAGTAGAGCGTCCTTATCAAGTGAAATAAA | 950 |
| SAUSA300_0648 (USA300-FPR3757) | GTTCTTTTGCTTTGCTGCTATAAGTAGAGCGTCCTTATCAAGTGAAATAAA | 950 |
| SAOUHSC_00668 (NCTC8325)       | GTTCTTTTGCTTTGCTGCTATAAGTAGAGCGTCCTTATCAAGTGAAATAAA | 860 |
| NWMN_0631 (Newman)             | GTTCTTTTGCTTTGCTGCTATAAGTAGAGCGTCCTTATCAAGTGAAATAAA | 815 |
| SAB0611 (RF122)                | GTTCTTTTGCTTTGCTGCTATAAGTAGAGCGTCCTTATCAAGTGAAATAAA | 950 |
|                                | *****                                               |     |

FIG. 23D

```
SaurJH1_0700 (JH1) ATATACTGCACCACACGACGTTACAATTAAGACCAACAAAAAGCTAATC 865
SaurJH9_0685 (JH9) ATATACTGCACCACACGACGTTACAATTAAGACCAACAAAAAGCTAATC 865
SAHV_0659 (Mu3) ATATACTGCACCACACGACGTTACAATTAAGACCAACAAAAAGCTAATC 865
SAV0662 (Mu50) ATATACTGCACCACACGACGTTACAATTAAGACCAACAAAAAGCTAATC 1000
SA0617 (N315) ATATACTGCACCACACGACGTTACAATTAAGACCAACAAAAAGCTAATC 1000
MW0624 (MW2) ATATACTGCACCACACGACGTTACAATTAAGACCAACAAAAAGCTAATC 1000
SAS0627 (MSSA476) ATATACTGCACCACACGACGTTACAATTAAGACCAACAAAAAGCTAATC 1000
SACOL0720_ ATATACTGCACCACACGACGTTACAATTAAGACCAACAAAAAGCTAATC 1000
SAUSA300_0648 (USA300-FPR3757) ATATACTGCACCACACGACGTTACAATTAAGACCAACAAAAAGCTAATC 1000
SAOUHSC_00668 (NCTC8325) ATATACTGCACCACACGACGTTACAATTAAGACCAACAAAAAGCTAATC 910
NWMN_0631 (Newman) ATATACTGCACCACACGACGTTACAATTAAGACCAACAAAAAGCTAATC 865
SAB0611 (RF122) ATATACTGCACCACACGACGTTACAATTAAGACCAACAAAAAGCTAATC 1000
*****

SaurJH1_0700 (JH1) AATTAGCAAGTGAATTAACAATCAAAAAATTCCTCATTTTATAATTAT 915
SaurJH9_0685 (JH9) AATTAGCAAGTGAATTAACAATCAAAAAATTCCTCATTTTATAATTAT 915
SAHV_0659 (Mu3) AATTAGCAAGTGAATTAACAATCAAAAAATTCCTCATTTTATAATTAT 915
SAV0662 (Mu50) AATTAGCAAGTGAATTAACAATCAAAAAATTCCTCATTTTATAATTAT 1050
SA0617 (N315) AATTAGCAAGTGAATTAACAATCAAAAAATTCCTCATTTTATAATTAT 1050
MW0624 (MW2) AATTAGCAAGTGAATTAACAATCAAAAAATTCCTCATTTTATAATTAT 1050
SAS0627 (MSSA476) AATTAGCAAGTGAATTAACAATCAAAAAATTCCTCATTTTATAATTAT 1050
SACOL0720_ AATTAGCAAGTGAATTAACAATCAAAAAATTCCTCATTTTATAATTAT 1050
SAUSA300_0648 (USA300-FPR3757) AATTAGCAAGTGAATTAACAATCAAAAAATTCCTCATTTTATAATTAT 1050
SAOUHSC_00668 (NCTC8325) AATTAGCAAGTGAATTAACAATCAAAAAATTCCTCATTTTATAATTAT 960
NWMN_0631 (Newman) AATTAGCAAGTGAATTAACAATCAAAAAATTCCTCATTTTATAATTAT 915
SAB0611 (RF122) AATTAGCAAGTGAATTAACAATCAAAAAATTCCTCATTTTATAATTAT 1050
*****

SaurJH1_0700 (JH1) AAAGAAGTAATTCATACGAAATTGTATAAAGATAATTTATTGATGTAAA 965
SaurJH9_0685 (JH9) AAAGAAGTAATTCATACGAAATTGTATAAAGATAATTTATTGATGTAAA 965
SAHV_0659 (Mu3) AAAGAAGTAATTCATACGAAATTGTATAAAGATAATTTATTGATGTAAA 965
SAV0662 (Mu50) AAAGAAGTAATTCATACGAAATTGTATAAAGATAATTTATTGATGTAAA 1100
SA0617 (N315) AAAGAAGTAATTCATACGAAATTGTATAAAGATAATTTATTGATGTAAA 1100
MW0624 (MW2) AAAGAAGTAATTCATACGAAATTGTATAAAGATAATTTATTGATGTAAA 1100
SAS0627 (MSSA476) AAAGAAGTAATTCATACGAAATTGTATAAAGATAATTTATTGATGTAAA 1100
SACOL0720_ AAAGAAGTAATTCATACGAAATTGTATAAAGATAATTTATTGATGTAAA 1100
SAUSA300_0648 (USA300-FPR3757) AAAGAAGTAATTCATACGAAATTGTATAAAGATAATTTATTGATGTAAA 1100
SAOUHSC_00668 (NCTC8325) AAAGAAGTAATTCATACGAAATTGTATAAAGATAATTTATTGATGTAAA 1010
NWMN_0631 (Newman) AAAGAAGTAATTCATACGAAATTGTATAAAGATAATTTATTGATGTAAA 965
SAB0611 (RF122) AAAGAAGTAATTCATACGAAATTGTATAAAGATAATTTATTGATGTAAA 1100
*****

SaurJH1_0700 (JH1) AGCGAAAGAACCATAACAATGTAACAATTACTAGTGATAAATATATCCCTA 1015
SaurJH9_0685 (JH9) AGCGAAAGAACCATAACAATGTAACAATTACTAGTGATAAATATATCCCTA 1015
SAHV_0659 (Mu3) AGCGAAAGAACCATAACAATGTAACAATTACTAGTGATAAATATATCCCTA 1015
SAV0662 (Mu50) AGCGAAAGAACCATAACAATGTAACAATTACTAGTGATAAATATATCCCTA 1150
SA0617 (N315) AGCGAAAGAACCATAACAATGTAACAATTACTAGTGATAAATATATCCCTA 1150
MW0624 (MW2) AGCGAAAGAACCATAACAATGTAACAATTACTAGTGATAAATATATCCCTA 1150
SAS0627 (MSSA476) AGCGAAAGAACCATAACAATGTAACAATTACTAGTGATAAATATATCCCTA 1150
SACOL0720_ AGCGAAAGAACCATAACAATGTAACAATTACTAGTGATAAATATATCCCTA 1150
SAUSA300_0648 (USA300-FPR3757) AGCGAAAGAACCATAACAATGTAACAATTACTAGTGATAAATATATCCCTA 1150
SAOUHSC_00668 (NCTC8325) AGCGAAAGAACCATAACAATGTAACAATTACTAGTGATAAATATATCCCTA 1060
NWMN_0631 (Newman) AGCGAAAGAACCATAACAATGTAACAATTACTAGTGATAAATATATCCCTA 1015
SAB0611 (RF122) ATCGAAACAACCATAACAATGTAACAATTACTAGTGATAAATATATCCCTA 1150
* * * * *

SaurJH1_0700 (JH1) ATACTGATTGAAACGTGGACAAGCTGATTGTTTGTAGCGGAAGGTTCT 1065
SaurJH9_0685 (JH9) ATACTGATTGAAACGTGGACAAGCTGATTGTTTGTAGCGGAAGGTTCT 1065
SAHV_0659 (Mu3) ATACTGATTGAAACGTGGACAAGCTGATTGTTTGTAGCGGAAGGTTCT 1065
SAV0662 (Mu50) ATACTGATTGAAACGTGGACAAGCTGATTGTTTGTAGCGGAAGGTTCT 1200
SA0617 (N315) ATACTGATTGAAACGTGGACAAGCTGATTGTTTGTAGCGGAAGGTTCT 1200
MW0624 (MW2) ATACTGATTGAAACGTGGACAAGCTGATTGTTTGTAGCGGAAGGTTCT 1200
SAS0627 (MSSA476) ATACTGATTGAAACGTGGACAAGCTGATTGTTTGTAGCGGAAGGTTCT 1200
SACOL0720_ ATACTGATTGAAACGTGGACAAGCTGATTGTTTGTAGCGGAAGGTTCT 1200
SAUSA300_0648 (USA300-FPR3757) ATACTGATTGAAACGTGGACAAGCTGATTGTTTGTAGCGGAAGGTTCT 1200
SAOUHSC_00668 (NCTC8325) ATACTGATTGAAACGTGGACAAGCTGATTGTTTGTAGCGGAAGGTTCT 1110
NWMN_0631 (Newman) ATACTGATTGAAACGTGGACAAGCTGATTGTTTGTAGCGGAAGGTTCT 1065
SAB0611 (RF122) GTACTGATTGAAACGTGGACAAGCTGATTGTTTGTAGCGGAAGGTTCT 1200
*****
```

FIG. 23E

|                                |                                                    |      |
|--------------------------------|----------------------------------------------------|------|
| SaurJH1_0700 (JH1)             | ATCAAAGATTTAGTGAAACATAAGAAGCATGGTAAGGCAATTATAGGAAC | 1115 |
| SaurJH9_0685 (JH9)             | ATCAAAGATTTAGTGAAACATAAGAAGCATGGTAAGGCAATTATAGGAAC | 1115 |
| SAHV_0659 (Mu3)                | ATCAAAGATTTAGTGAAACATAAGAAGCATGGTAAGGCAATTATAGGAAC | 1115 |
| SAV0662 (Mu50)                 | ATCAAAGATTTAGTGAAACATAAGAAGCATGGTAAGGCAATTATAGGAAC | 1250 |
| SA0617 (N315)                  | ATCAAAGATTTAGTGAAACATAAGAAGCATGGTAAGGCAATTATAGGAAC | 1250 |
| MW0624 (MW2)                   | ATCAAAGATTTAGTGAAACATAAGAAGCATGGTAAGGCAATTATAGGAAC | 1250 |
| SAS0627 (MSSA476)              | ATCAAAGATTTAGTGAAACATAAGAAGCATGGTAAGGCAATTATAGGAAC | 1250 |
| SACOL0720_                     | ATCAAAGATTTAGTGAAACATAAGAAGCATGGTAAGGCAATTATAGGAAC | 1250 |
| SAUSA300_0648 (USA300-FPR3757) | ATCAAAGATTTAGTGAAACATAAGAAGCATGGTAAGGCAATTATAGGAAC | 1250 |
| SAOUHSC_00668 (NCTC8325)       | ATCAAAGATTTAGTGAAACATAAGAAGCATGGTAAGGCAATTATAGGAAC | 1160 |
| NWMN_0631 (Newman)             | ATCAAAGATTTAGTGAAACATAAGAAGCATGGTAAGGCAATTATAGGAAC | 1115 |
| SAB0611 (RF122)                | ATCAAAGATTTAGTGAAACATAAGAAGCATGGTAAGGCAATTATAGGAAC | 1250 |
|                                | *****                                              |      |
| SaurJH1_0700 (JH1)             | GAAAAACATCATGTTAATATTAAGTTACGGAAGATATTAATAAAATCT   | 1165 |
| SaurJH9_0685 (JH9)             | GAAAAACATCATGTTAATATTAAGTTACGGAAGATATTAATAAAATCT   | 1165 |
| SAHV_0659 (Mu3)                | GAAAAACATCATGTTAATATTAAGTTACGGAAGATATTAATAAAATCT   | 1165 |
| SAV0662 (Mu50)                 | GAAAAACATCATGTTAATATTAAGTTACGGAAGATATTAATAAAATCT   | 1300 |
| SA0617 (N315)                  | GAAAAACATCATGTTAATATTAAGTTACGGAAGATATTAATAAAATCT   | 1300 |
| MW0624 (MW2)                   | GAAAAACATCATGTTAATATTAAGTTGCGGAAGATATTAATAAAATCT   | 1300 |
| SAS0627 (MSSA476)              | GAAAAACATCATGTTAATATTAAGTTGCGGAAGATATTAATAAAATCT   | 1300 |
| SACOL0720_                     | GAAAAACATCATGTTAATATTAAGTTACGTAAGATATTAATAAAATCT   | 1300 |
| SAUSA300_0648 (USA300-FPR3757) | GAAAAACATCATGTTAATATTAAGTTACGTAAGATATTAATAAAATCT   | 1300 |
| SAOUHSC_00668 (NCTC8325)       | GAAAAACATCATGTTAATATTAAGTTACGTAAGATATTAATAAAATCT   | 1210 |
| NWMN_0631 (Newman)             | GAAAAACATCATGTTAATATTAAGTTACGTAAGATATTAATAAAATCT   | 1165 |
| SAB0611 (RF122)                | GAAAAACATCATGTTAATATTAAGTTACGTAAGATATTAATAAAATCT   | 1300 |
|                                | *****                                              |      |
| SaurJH1_0700 (JH1)             | ATTTTATGACAGATGTTGATTAGGTGGACCAACGTTTGTCTTAAATGAC  | 1215 |
| SaurJH9_0685 (JH9)             | ATTTTATGACAGATGTTGATTAGGTGGACCAACGTTTGTCTTAAATGAC  | 1215 |
| SAHV_0659 (Mu3)                | ATTTTATGACAGATGTTGATTAGGTGGACCAACGTTTGTCTTAAATGAC  | 1215 |
| SAV0662 (Mu50)                 | ATTTTATGACAGATGTTGATTAGGTGGACCAACGTTTGTCTTAAATGAC  | 1350 |
| SA0617 (N315)                  | ATTTTATGACAGATGTTGATTAGGTGGACCAACGTTTGTCTTAAATGAC  | 1350 |
| MW0624 (MW2)                   | ATTTTATGACAGATGTTGATTAGGTGGACCAACGTTTGTCTTAAATGAC  | 1350 |
| SAS0627 (MSSA476)              | ATTTTATGACAGATGTTGATTAGGTGGACCAACGTTTGTCTTAAATGAC  | 1350 |
| SACOL0720_                     | ATTTTATGACAGATGTTGATTAGGTGGACCAACGTTTGTCTTAAATGAC  | 1350 |
| SAUSA300_0648 (USA300-FPR3757) | ATTTTATGACAGATGTTGATTAGGTGGACCAACGTTTGTCTTAAATGAC  | 1350 |
| SAOUHSC_00668 (NCTC8325)       | ATTTTATGACAGATGTTGATTAGGTGGACCAACGTTTGTCTTAAATGAC  | 1260 |
| NWMN_0631 (Newman)             | ATTTTATGACAGATGTTGATTAGGTGGACCAACGTTTGTCTTAAATGAC  | 1215 |
| SAB0611 (RF122)                | ATTTTATGACAGATGTTGATTAGGTGGACCAACGTTTGTCTTAAATGAC  | 1350 |
|                                | *****                                              |      |
| SaurJH1_0700 (JH1)             | AAAGACTATCAAGAAATAAGAAAGTATACAAAAGCAAAGCATATCGTCTC | 1265 |
| SaurJH9_0685 (JH9)             | AAAGACTATCAAGAAATAAGAAAGTATACAAAAGCAAAGCATATCGTCTC | 1265 |
| SAHV_0659 (Mu3)                | AAAGACTATCAAGAAATAAGAAAGTATACAAAAGCAAAGCATATCGTCTC | 1265 |
| SAV0662 (Mu50)                 | AAAGACTATCAAGAAATAAGAAAGTATACAAAAGCAAAGCATATCGTCTC | 1400 |
| SA0617 (N315)                  | AAAGACTATCAAGAAATAAGAAAGTATACAAAAGCAAAGCATATCGTCTC | 1400 |
| MW0624 (MW2)                   | AAAGACTATCAAGAAATAAGAAAGTATACAAAAGCAAAGCATATCGTCTC | 1400 |
| SAS0627 (MSSA476)              | AAAGACTATCAAGAAATAAGAAAGTATACAAAAGCAAAGCATATCGTCTC | 1400 |
| SACOL0720_                     | AAAGACTATCAAGAAATAAGAAAGTATACAAAAGCAAAGCATATCGTCTC | 1400 |
| SAUSA300_0648 (USA300-FPR3757) | AAAGACTATCAAGAAATAAGAAAGTATACAAAAGCAAAGCATATCGTCTC | 1400 |
| SAOUHSC_00668 (NCTC8325)       | AAAGACTATCAAGAAATAAGAAAGTATACAAAAGCAAAGCATATCGTCTC | 1310 |
| NWMN_0631 (Newman)             | AAAGACTATCAAGAAATAAGAAAGTATACAAAAGCAAAGCATATCGTCTC | 1265 |
| SAB0611 (RF122)                | AAAGACTATCAAGAAATAAGAAAGTATACAAAAGCAAAGCATATCGTCTC | 1400 |
|                                | *****                                              |      |
| SaurJH1_0700 (JH1)             | TCAATTTGGATTTCGATTGAAACATAAAAAAGATGCTTTAGCATTAGAAA | 1315 |
| SaurJH9_0685 (JH9)             | TCAATTTGGATTTCGATTGAAACATAAAAAAGATGCTTTAGCATTAGAAA | 1315 |
| SAHV_0659 (Mu3)                | TCAATTTGGATTTCGATTGAAACATAAAAAAGATGCTTTAGCATTAGAAA | 1315 |
| SAV0662 (Mu50)                 | TCAATTTGGATTTCGATTGAAACATAAAAAAGATGCTTTAGCATTAGAAA | 1450 |
| SA0617 (N315)                  | TCAATTTGGATTTCGATTGAAACATAAAAAAGATGCTTTAGCATTAGAAA | 1450 |
| MW0624 (MW2)                   | TCAATTTGGATTTCGATTGAAACATAAAAAAGATGCTTTAGCATTAGAAA | 1450 |
| SAS0627 (MSSA476)              | TCAATTTGGATTTCGATTGAAACATAAAAAAGATGCTTTAGCATTAGAAA | 1450 |
| SACOL0720_                     | TCAATTTGGATTTCGATTGAAACATAAAAAAGATGCTTTAGCATTAGAAA | 1450 |
| SAUSA300_0648 (USA300-FPR3757) | TCAATTTGGATTTCGATTGAAACATAAAAAAGATGCTTTAGCATTAGAAA | 1450 |
| SAOUHSC_00668 (NCTC8325)       | TCAATTTGGATTTCGATTGAAACATAAAAAAGATGCTTTAGCATTAGAAA | 1360 |
| NWMN_0631 (Newman)             | TCAATTTGGATTTCGATTGAAACATAAAAAAGATGCTTTAGCATTAGAAA | 1315 |
| SAB0611 (RF122)                | TCAATTTGGATTTCGATTGAAACATAAAAAAGATGCTTTAGCATTAGAAA | 1450 |
|                                | *****                                              |      |

FIG. 23F

|                                |                                                      |      |
|--------------------------------|------------------------------------------------------|------|
| SaurJH1_0700 (JH1)             | AAGTGAAAAATAAAGTTGATAAATCTATTAAAAACAAGAAGTGAAGCGATA  | 1365 |
| SaurJH9_0685 (JH9)             | AAGTGAAAAATAAAGTTGATAAATCTATTAAAAACAAGAAGTGAAGCGATA  | 1365 |
| SAHV_0659 (Mu3)                | AAGTGAAAAATAAAGTTGATAAATCTATTAAAAACAAGAAGTGAAGCGATA  | 1365 |
| SAV0662 (Mu50)                 | AAGTGAAAAATAAAGTTGATAAATCTATTAAAAACAAGAAGTGAAGCGATA  | 1500 |
| SA0617 (N315)                  | AAGTGAAAAATAAAGTTGATAAATCTATTAAAAACAAGAAGTGAAGCGATA  | 1500 |
| MW0624 (MW2)                   | AAGTGAAAAATAAAGTTGATAAATCTATTAAAAACAAGAAGTGAAGCGATA  | 1500 |
| SAS0627 (MSSA476)              | AAGTGAAAAATAAAGTTGATAAATCTATTAAAAACAAGAAGTGAAGCGATA  | 1500 |
| SACOL0720_                     | AAGCGAAAAATAAAGTTGATAAATCTATTGAAACAAGAAGTGAAGCGATA   | 1500 |
| SAUSA300_0648 (USA300-FPR3757) | AAGCGAAAAATAAAGTTGATAAATCTATTGAAACAAGAAGTGAAGCGATA   | 1500 |
| SAOUHSC_00668 (NCTC8325)       | AAGCGAAAAATAAAGTTGATAAATCTATTGAAACAAGAAGTGAAGCGATA   | 1410 |
| NWMN_0631 (Newman)             | AAGCGAAAAATAAAGTTGATAAATCTATTGAAACAAGAAGTGAAGCGATA   | 1365 |
| SAB0611 (RF122)                | AAGCGAAAAATAAAGTTGATAAATCTATTGAGACAAGAAGTGAAGCGATA   | 1500 |
|                                | *** ***** * *****                                    |      |
| SaurJH1_0700 (JH1)             | AGCTCAATATCAAGTTTAAACCGGAATATTATTATTTGTAACATCATTTTT  | 1415 |
| SaurJH9_0685 (JH9)             | AGCTCAATATCAAGTTTAAACCGGAATATTATTATTTGTAACATCATTTTT  | 1415 |
| SAHV_0659 (Mu3)                | AGCTCAATATCAAGTTTAAACCGGAATATTATTATTTGTAACATCATTTTT  | 1415 |
| SAV0662 (Mu50)                 | AGCTCAATATCAAGTTTAAACCGGAATATTATTATTTGTAACATCATTTTT  | 1550 |
| SA0617 (N315)                  | AGCTCAATATCAAGTTTAAACCGGAATATTATTATTTGTAACATCATTTTT  | 1550 |
| MW0624 (MW2)                   | AGCTCAATATCAAGTTTAAACCGGAATATTATTATTTGTAACATCATTTTT  | 1550 |
| SAS0627 (MSSA476)              | AGCTCAATATCAAGTTTAAACCGGAATATTATTATTTGTAACATCATTTTT  | 1550 |
| SACOL0720_                     | AGCTCAATATCAAGTTTAAACCGGAATATTATTATTTGTAACATCATTTTT  | 1550 |
| SAUSA300_0648 (USA300-FPR3757) | AGCTCAATATCAAGTTTAAACCGGAATATTATTATTTGTAACATCATTTTT  | 1550 |
| SAOUHSC_00668 (NCTC8325)       | AGCTCAATATCAAGTTTAAACCGGAATATTATTATTTGTAACATCATTTTT  | 1460 |
| NWMN_0631 (Newman)             | AGCTCAATATCAAGTTTAAACCGGAATATTATTATTTGTAACATCATTTTT  | 1415 |
| SAB0611 (RF122)                | AGCTCAATATCAAGTTTAAACCGGAATATTATTATTTGTAACATCATTTTT  | 1550 |
|                                | *****                                                |      |
| SaurJH1_0700 (JH1)             | AGGTATTACATTCCTTGATTGCTGTATGTTGCATTATATACATTAAAGCAAA | 1465 |
| SaurJH9_0685 (JH9)             | AGGTATTACATTCCTTGATTGCTGTATGTTGCATTATATACATTAAAGCAAA | 1465 |
| SAHV_0659 (Mu3)                | AGGTATTACATTCCTTGATTGCTGTATGTTGCATTATATACATTAAAGCAAA | 1465 |
| SAV0662 (Mu50)                 | AGGTATTACATTCCTTGATTGCTGTATGTTGCATTATATACATTAAAGCAAA | 1600 |
| SA0617 (N315)                  | AGGTATTACATTCCTTGATTGCTGTATGTTGCATTATATACATTAAAGCAAA | 1600 |
| MW0624 (MW2)                   | AGGTATTACATTCCTTGATTGCTGTATGTTGCATTATATACATTAAAGCAAA | 1600 |
| SAS0627 (MSSA476)              | AGGTATTACATTCCTTGATTGCTGTATGTTGCATTATATACATTAAAGCAAA | 1600 |
| SACOL0720_                     | AGGTATTACATTCCTTGATTGCTGTATGTTGCATTATATACATTAAAGCAAA | 1600 |
| SAUSA300_0648 (USA300-FPR3757) | AGGTATTACATTCCTTGATTGCTGTATGTTGCATTATATACATTAAAGCAAA | 1600 |
| SAOUHSC_00668 (NCTC8325)       | AGGTATTACATTCCTTGATTGCTGTATGTTGCATTATATACATTAAAGCAAA | 1510 |
| NWMN_0631 (Newman)             | AGGTATTACATTCCTTGATTGCTGTATGTTGCATTATATACATTAAAGCAAA | 1465 |
| SAB0611 (RF122)                | AGGTATTACATTCCTTGATTGCTGTATGTTGCATTATATACATTAAAGCAAA | 1600 |
|                                | *****                                                |      |
| SaurJH1_0700 (JH1)             | TAGATGAAACCGAAGATGAGTTAGAGAATTATAGTATATTGAGAAAGCTT   | 1515 |
| SaurJH9_0685 (JH9)             | TAGATGAAACCGAAGATGAGTTAGAGAATTATAGTATATTGAGAAAGCTT   | 1515 |
| SAHV_0659 (Mu3)                | TAGATGAAACCGAAGATGAGTTAGAGAATTATAGTATATTGAGAAAGCTT   | 1515 |
| SAV0662 (Mu50)                 | TAGATGAAACCGAAGATGAGTTAGAGAATTATAGTATATTGAGAAAGCTT   | 1650 |
| SA0617 (N315)                  | TAGATGAAACCGAAGATGAGTTAGAGAATTATAGTATATTGAGAAAGCTT   | 1650 |
| MW0624 (MW2)                   | TAGATGAAACCGAAGATGAGTTAGAGAATTATAGTATATTGAGAAAGCTT   | 1650 |
| SAS0627 (MSSA476)              | TAGATGAAACCGAAGATGAGTTAGAGAATTATAGTATATTGAGAAAGCTT   | 1650 |
| SACOL0720_                     | TAGATGAAACCGAAGATGAGTTAGAGAATTATAGTATATTGAGAAAGCTT   | 1650 |
| SAUSA300_0648 (USA300-FPR3757) | TAGATGAAACCGAAGATGAGTTAGAGAATTATAGTATATTGAGAAAGCTT   | 1650 |
| SAOUHSC_00668 (NCTC8325)       | TAGATGAAACCGAAGATGAGTTAGAGAATTATAGTATATTGAGAAAGCTT   | 1560 |
| NWMN_0631 (Newman)             | TAGATGAAACCGAAGATGAGTTAGAGAATTATAGTATATTGAGAAAGCTT   | 1515 |
| SAB0611 (RF122)                | TAGATGAAACCGAAGATGAGTTAGAGAATTATAGTATATTGAGAAAGCTT   | 1650 |
|                                | *****                                                |      |
| SaurJH1_0700 (JH1)             | GGATTTACACAAAAAGATATGGCAAGGGGACTAAAGTTTAAAAATTATGTT  | 1565 |
| SaurJH9_0685 (JH9)             | GGATTTACACAAAAAGATATGGCAAGGGGACTAAAGTTTAAAAATTATGTT  | 1565 |
| SAHV_0659 (Mu3)                | GGATTTACACAAAAAGATATGGCAAGGGGACTAAAGTTTAAAAATTATGTT  | 1565 |
| SAV0662 (Mu50)                 | GGATTTACACAAAAAGATATGGCAAGGGGACTAAAGTTTAAAAATTATGTT  | 1700 |
| SA0617 (N315)                  | GGATTTACACAAAAAGATATGGCAAGGGGACTAAAGTTTAAAAATTATGTT  | 1700 |
| MW0624 (MW2)                   | GGATTTACACAAAAAGATATGGCAAGGGGACTAAAGTTTAAAAATTATGTT  | 1700 |
| SAS0627 (MSSA476)              | GGATTTACACAAAAAGATATGGCAAGGGGACTAAAGTTTAAAAATTATGTT  | 1700 |
| SACOL0720_                     | GGATTTACACAAAAAGATATGGCAAGGGGACTAAAGTTTAAAAATTATGTT  | 1700 |
| SAUSA300_0648 (USA300-FPR3757) | GGATTTACACAAAAAGATATGGCAAGGGGACTAAAGTTTAAAAATTATGTT  | 1700 |
| SAOUHSC_00668 (NCTC8325)       | GGATTTACACAAAAAGATATGGCAAGGGGACTAAAGTTTAAAAATTATGTT  | 1610 |
| NWMN_0631 (Newman)             | GGATTTACACAAAAAGATATGGCAAGGGGACTAAAGTTTAAAAATTATGTT  | 1565 |
| SAB0611 (RF122)                | GGATTTACACAAAAAGATATGGCAAGGGGACTAAAGTTTAAAAATTATGTT  | 1700 |
|                                | *****                                                |      |

FIG. 23G

|                                |                                                     |      |
|--------------------------------|-----------------------------------------------------|------|
| SaurJH1_0700 (JH1)             | TAATTTTGGGTTACCTTTAGTTATTGCACTATCACATGCATATTTTACAT  | 1615 |
| SaurJH9_0685 (JH9)             | TAATTTTGGGTTACCTTTAGTTATTGTACTATCACATGCATATTTTACAT  | 1615 |
| SAHV_0659 (Mu3)                | TAATTTTGGGTTACCTTTAGTTATTGCACTATCACATGCATATTTTACAT  | 1615 |
| SAV0662 (Mu50)                 | TAATTTTGGGTTACCTTTAGTTATTGCACTATCACATGCATATTTTACAT  | 1750 |
| SA0617 (N315)                  | TAATTTTGGGTTACCTTTAGTTATTGCACTATCACATGCATATTTTACAT  | 1750 |
| MW0624 (MW2)                   | TAATTTTGGGTTACCTTTAGTTATTGCACTATCACATGCATATTTTACAT  | 1750 |
| SAS0627 (MSSA476)              | TAATTTTGGGTTACCTTTAGTTATTGCACTATCACATGCATATTTTACAT  | 1750 |
| SACOL0720_                     | TAATTTTGGGTTACCTTTAGTTATTGCACTATCACATGCATATTTTACAT  | 1750 |
| SAUSA300_0648 (USA300-FPR3757) | TAATTTTGGGTTACCTTTAGTTATTGCACTATCACATGCATATTTTACAT  | 1750 |
| SAOUHSC_00668 (NCTC8325)       | TAATTTTGGGTTACCTTTAGTTATTGCACTATCACATGCATATTTTACAT  | 1660 |
| NWMN_0631 (Newman)             | TAATTTTGGGTTACCTTTAGTTATTGCACTATCACATGCATATTTTACAT  | 1615 |
| SAB0611 (RF122)                | TAATTTTGGGTTACCTTTAGTTATTGCACTATCACATGCATATTTTACAT  | 1750 |
|                                | *****                                               |      |
| SaurJH1_0700 (JH1)             | CATTAGCATATATGAAATTAATGGGTACAACGAATCAAATACCGGTTTTTC | 1665 |
| SaurJH9_0685 (JH9)             | CATTAGCATATATGAAATTAATGGGTACAACGAATCAAATACCGGTTTTTC | 1665 |
| SAHV_0659 (Mu3)                | CATTAGCATATATGAAATTAATGGGTACAACGAATCAAATACCGGTTTTTC | 1665 |
| SAV0662 (Mu50)                 | CATTAGCATATATGAAATTAATGGGTACAACGAATCAAATACCGGTTTTTC | 1800 |
| SA0617 (N315)                  | CATTAGCATATATGAAATTAATGGGTACAACGAATCAAATACCGGTTTTTC | 1800 |
| MW0624 (MW2)                   | CATTAGCATATATGAAATTAATGGGTACAACGAATCAAATACCGGTTTTTC | 1800 |
| SAS0627 (MSSA476)              | CATTAGCATATATGAAATTAATGGGTACAACGAATCAAATACCGGTTTTTC | 1800 |
| SACOL0720_                     | CATTAGCATATATGAAATTAATGGGTACAACGAATCAAATACCGGTTTTTC | 1800 |
| SAUSA300_0648 (USA300-FPR3757) | CATTAGCATATATGAAATTAATGGGTACAACGAATCAAATACCGGTTTTTC | 1800 |
| SAOUHSC_00668 (NCTC8325)       | CATTAGCATATATGAAATTAATGGGTACAACGAATCAAATACCGGTTTTTC | 1710 |
| NWMN_0631 (Newman)             | CATTAGCATATATGAAATTAATGGGTACAACGAATCAAATACCGGTTTTTC | 1665 |
| SAB0611 (RF122)                | CATTAGCATATATGAAATTAATGGGTACAACGAATCAAATACCGGTTTTTC | 1800 |
|                                | *****                                               |      |
| SaurJH1_0700 (JH1)             | ATAGTAATGGGATTATACATTTGTATGTATGCTGTTTTTGCAGTGACGGC  | 1715 |
| SaurJH9_0685 (JH9)             | ATAGTAATGGGATTATACATTTGTATGTATGCTGTTTTTGCAGTGACGGC  | 1715 |
| SAHV_0659 (Mu3)                | ATAGTAATGGGATTATACATTTGTATGTATGCTGTTTTTGCAGTGACGGC  | 1715 |
| SAV0662 (Mu50)                 | ATAGTAATGGGATTATACATTTGTATGTATGCTGTTTTTGCAGTGACGGC  | 1850 |
| SA0617 (N315)                  | ATAGTAATGGGATTATACATTTGTATGTATGCTGTTTTTGCAGTGACGGC  | 1850 |
| MW0624 (MW2)                   | ATAGTAATGGGATTATACATTTGTATGTATGCTGTTTTTGCAGTGACGGC  | 1850 |
| SAS0627 (MSSA476)              | ATAGTAATGGGATTATACATTTGTATGTATGCTGTTTTTGCAGTGACGGC  | 1850 |
| SACOL0720_                     | ATAGTAATGGGATTATACATTTGTATGTATGCTGTTTTTGCAGTGACGGC  | 1850 |
| SAUSA300_0648 (USA300-FPR3757) | ATAGTAATGGGATTATACATTTGTATGTATGCTGTTTTTGCAGTGACGGC  | 1850 |
| SAOUHSC_00668 (NCTC8325)       | ATAGTAATGGGATTATACATTTGTATGTATGCTGTTTTTGCAGTGACGGC  | 1760 |
| NWMN_0631 (Newman)             | ATAGTAATGGGATTATACATTTGTATGTATGCTGTTTTTGCAGTGACGGC  | 1715 |
| SAB0611 (RF122)                | ATAGTAATGGGATTATACATTTGTATGTATGCTGTTTTTGCAGTGACGGC  | 1850 |
|                                | *****                                               |      |
| SaurJH1_0700 (JH1)             | TTATAATCATTCCTCAAGCGAACAATTAGACATTCCATATAA          | 1755 |
| SaurJH9_0685 (JH9)             | TTATAATCATTCCTCAAGCGAACAATTAGACATTCCATATAA          | 1755 |
| SAHV_0659 (Mu3)                | TTATAATCATTCCTCAAGCGAACAATTAGACATTCCATATAA          | 1755 |
| SAV0662 (Mu50)                 | TTATAATCATTCCTCAAGCGAACAATTAGACATTCCATATAA          | 1890 |
| SA0617 (N315)                  | TTATAATCATTCCTCAAGCGAACAATTAGACATTCCATATAA          | 1890 |
| MW0624 (MW2)                   | TTATAATCATTCCTCAAGCGAACAATTAGACATTCCATATAA          | 1890 |
| SAS0627 (MSSA476)              | TTATAATCATTCCTCAAGCGAACAATTAGACATTCCATATAA          | 1890 |
| SACOL0720_                     | TTATAATCATTCCTCAAGCGAACAATTAGACATTCCATATAA          | 1890 |
| SAUSA300_0648 (USA300-FPR3757) | TTATAATCATTCCTCAAGCGAACAATTAGACATTCCATATAA          | 1890 |
| SAOUHSC_00668 (NCTC8325)       | TTATAATCATTCCTCAAGCGAACAATTAGACATTCCATATAA          | 1800 |
| NWMN_0631 (Newman)             | TTATAATCATTCCTCAAGCGAACAATTAGACATTCCATATAA          | 1755 |
| SAB0611 (RF122)                | TTATAATCATTCCTCAAGCGAACAATTAGACATTCCATATAA          | 1890 |
|                                | *****                                               |      |

FIG. 23H

**II- SACOL0720 : Multiple polypeptide sequences alignment: selected epitopes shaded**

|                                |                                                     |     |
|--------------------------------|-----------------------------------------------------|-----|
| SACOL0720_                     | MTFNEIIFKNFRQNLSHYAIYLFSLITSVVLYFSFVALKYAHKLNMTESY  | 50  |
| SAUSA300_0648 (USA300-FPR3757) | MTFNEIIFKNFRQNLSHYAIYLFSLITSVVLYFSFVALKYAHKLNMTESY  | 50  |
| SAOUHSC_00668 (NCTC8325)       | -----MYFSFVALKYAHKLNMTESY                           | 20  |
| NWMN_0631 (Newman)             | -----MTESY                                          | 5   |
| SaurJH1_0700 (JH1)             | -----MTESY                                          | 5   |
| SaurJH9_0685 (JH9)             | -----MTESY                                          | 5   |
| SAHV_0659 (Mu3)                | -----MTESY                                          | 5   |
| SAV0662 (Mu50)                 | MTFNEIIFKNFRQNLSHYAIYLFSLITSVVLYFSFVALKYAHKLNMTESY  | 50  |
| SA0617 (N315)                  | MTFNEIIFKNFRQNLSHYAIYLFSLITSVVLYFSFVALKYAHKLNMTESY  | 50  |
| MW0624 (MW2)                   | MTFNEIIFKNFRQNLSHYAIYLFSLITSVVLYFSFVALKYAHKLNMTESY  | 50  |
| SAS0627 (MSSA476)              | MTFNEIIFKNFRQNLSHYAIYLFSLITSVVLYFSFVALKYAHKLNMTESY  | 50  |
| SAB0611 (RF122)                | MTFNEIIFKNFRQNLSHYAIYLFSLITSVVLYFSFVALKYAHKLNMTESY  | 50  |
|                                | *****                                               |     |
| Consensus                      | XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMTESY   |     |
| SACOL0720_                     | PIIKEGSQVGSYFLFFIIIAFLLYANVLFIKRRSYELALYQTLGLSKFNI  | 100 |
| SAUSA300_0648 (USA300-FPR3757) | PIIKEGSQVGSYFLFFIIIAFLLYANVLFIKRRSYELALYQTLGLSKFNI  | 100 |
| SAOUHSC_00668 (NCTC8325)       | PIIKEGSQVGSYFLFFIIIAFLLYANVLFIKRRSYELALYQTLGLSKFNI  | 70  |
| NWMN_0631 (Newman)             | PIIKEGSQVGSYFLFFIIIAFLLYANVLFIKRRSYELALYQTLGLSKFNI  | 55  |
| SaurJH1_0700 (JH1)             | PIIKEGSQVGSYFLFFIIIAFLLYANVLFIKRRSYELALYQTLGLSKFNI  | 55  |
| SaurJH9_0685 (JH9)             | PIIKEGSQVGSYFLFFIIIAFLLYANVLFIKRRSYELALYQTLGLSKFNI  | 55  |
| SAHV_0659 (Mu3)                | PIIKEGSQVGSYFLFFIIIAFLLYANVLFIKRRSYELALYQTLGLSKFNI  | 55  |
| SAV0662 (Mu50)                 | PIIKEGSQVGSYFLFFIIIAFLLYANVLFIKRRSYELALYQTLGLSKFNI  | 100 |
| SA0617 (N315)                  | PIIKEGSQVGSYFLFFIIIAFLLYANVLFIKRRSYELALYQTLGLSKFNI  | 100 |
| MW0624 (MW2)                   | PIIKEGSQVGSYFLFFIIIAFLLYANVLFIKRRSYELALYQTLGLSKFNI  | 100 |
| SAS0627 (MSSA476)              | PIIKEGSQVGSYFLFFIIIAFLLYANVLFIKRRSYELALYQTLGLSKFNI  | 100 |
| SAB0611 (RF122)                | PIIKEGSQVGSYFLFFIIIAFLLYANVLFIKRRSYELALYQTLGLSKFNI  | 100 |
|                                | *****                                               |     |
| Consensus                      | PIIKEGSQVGSYFLFFIIIAFLLYANVLFIKRRSYELALYQTLGLSKFNI  |     |
| SACOL0720_                     | IYILMLEQLLIFIITAILGIIIGIFGSKLLLMIVFTLLGIKEKVPPIIFSL | 150 |
| SAUSA300_0648 (USA300-FPR3757) | IYILMLEQLLIFIITAILGIIIGIFGSKLLLMIVFTLLGIKEKVPPIIFSL | 150 |
| SAOUHSC_00668 (NCTC8325)       | IYILMLEQLLIFIITAILGIIIGIFGSKLLLMIVFTLLGIKEKVPPIIFSL | 120 |
| NWMN_0631 (Newman)             | IYILMLEQLLIFIITAILGIIIGIFGSKLLLMIVFTLLGIKEKVPPIIFSL | 105 |
| SaurJH1_0700 (JH1)             | IYILMLEQLLIFIITAILGIIIGIFGSKLLLMIVFTLLGIKEKVPPIIFSL | 105 |
| SaurJH9_0685 (JH9)             | IYILMLEQLLIFIITAILGIIIGIFGSKLLLMIVFTLLGIKEKVPPIIFSL | 105 |
| SAHV_0659 (Mu3)                | IYILMLEQLLIFIITAILGIIIGIFGSKLLLMIVFTLLGIKEKVPPIIFSL | 105 |
| SAV0662 (Mu50)                 | IYILMLEQLLIFIITAILGIIIGIFGSKLLLMIVFTLLGIKEKVPPIIFSL | 150 |
| SA0617 (N315)                  | IYILMLEQLLIFIITAILGIIIGIFGSKLLLMIVFTLLGIKEKVPPIIFSL | 150 |
| MW0624 (MW2)                   | IYILMLEQLLIFIITAILGIIIGIFGSKLLLMIVFTLLGIKEKVPPIIFSL | 150 |
| SAS0627 (MSSA476)              | IYILMLEQLLIFIITAILGIIIGIFGSKLLLMIVFTLLGIKEKVPPIIFSL | 150 |
| SAB0611 (RF122)                | IYILMLEQLLIFIITAILGIIIGIFGSKLLLMIVFTLLGIKEKVPPIIFSL | 150 |
|                                | *****                                               |     |
| Consensus                      | IYILMLEQLLIFIITAILGIIIGIFGSKLLLMIVFTLLGIKEKVPPIIFSL |     |
| SACOL0720_                     | RAVFETLMLIGVAYFLTSAQNFILVFKQSIQMSKNNQVKE TNHNKI TFE | 200 |
| SAUSA300_0648 (USA300-FPR3757) | RAVFETLMLIGVAYFLTSAQNFILVFKQSIQMSKNNQVKE TNHNKI TFE | 200 |
| SAOUHSC_00668 (NCTC8325)       | RAVFETLMLIGVAYFLTSAQNFILVFKQSIQMSKNNQVKE TNHNKI TFE | 170 |
| NWMN_0631 (Newman)             | RAVFETLMLIGVAYFLTSAQNFILVFKQSIQMSKNNQVKE TNHNKI TFE | 155 |
| SaurJH1_0700 (JH1)             | RAVFETLMLIGVAYFLTSAQNFILVFKQSIQMSKNNQVKE TNHNKI TFE | 155 |
| SaurJH9_0685 (JH9)             | RAVFETLMLIGVAYFLTSAQNFILVFKQSIQMSKNNQVKE TNHNKI TFE | 155 |
| SAHV_0659 (Mu3)                | RAVFETLMLIGVAYFLTSAQNFILVFKQSIQMSKNNQVKE TNHNKI TFE | 155 |
| SAV0662 (Mu50)                 | RAVFETLMLIGVAYFLTSAQNFILVFKQSIQMSKNNQVKE TNHNKI TFE | 200 |
| SA0617 (N315)                  | RAVFETLMLIGVAYFLTSAQNFILVFKQSIQMSKNNQVKE TNHNKI TFE | 200 |
| MW0624 (MW2)                   | RAVFETLMLIGVAYFLTSAQNFILVFKQSIQMSKNNQVKE TNHNKI TFE | 200 |
| SAS0627 (MSSA476)              | RAVFETLMLIGVAYFLTSAQNFILVFKQSIQMSKNNQVKE TNHNKI TFE | 200 |
| SAB0611 (RF122)                | RAVFETLMLIGVAYFLTSAQNFILVFKQSIQMSKNNQVKE TNHNKI TFE | 200 |
|                                | *****                                               |     |
| Consensus                      | RAVFETLMLIGVAYFLTSAQNFILVFKQSIQMSKNNQVKE TNHNKI TFE |     |

**FIG. 231**

|                                |                                                     |     |
|--------------------------------|-----------------------------------------------------|-----|
| SACOL0720_                     | EVVLGILGIVLIITGGYLSLNIVQYYDSIGTLMFILLSTVIGAYLFFKSS  | 250 |
| SAUSA300_0648 (USA300-FPR3757) | EVVLGILGIVLIITGGYLSLNIVQYYDSIGTLMFILLSTVIGAYLFFKSS  | 250 |
| SAOUHSC_00668 (NCTC8325)       | EVVLGILGIVLIITGGYLSLNIVQYYDSIGTLMFILLSTVIGAYLFFKSS  | 220 |
| NWMN_0631 (Newman)             | EVVLGILGIVLIITGGYLSLNIVQYYDSIGTLMFILLSTVIGAYLFFKSS  | 205 |
| SaurJH1_0700 (JH1)             | EVVLGILGIVLIITGGYLSLNIVQYYDSIGTLMFILLSTVIGAYLFFKSS  | 205 |
| SaurJH9_0685 (JH9)             | EVVLGILGIVLIITGGYLSLNIVQYYDSIGTLMFILLSTVIGAYLFFKSS  | 205 |
| SAHV_0659 (Mu3)                | EVVLGILGIVLIITGGYLSLNIVQYYDSIGTLMFILLSTVIGAYLFFKSS  | 205 |
| SAV0662 (Mu50)                 | EVVLGILGIVLIITGGYLSLNIVQYYDSIGTLMFILLSTVIGAYLFFKSS  | 250 |
| SA0617 (N315)                  | EVVLGILGIVLIITGGYLSLNIVQYYDSIGTLMFILLSTVIGAYLFFKSS  | 250 |
| MW0624 (MW2)                   | EVVLGILGIVLIITGGYLSLNIVQYYDSIGTLMFILLSTVIGAYLFFKSS  | 250 |
| SAS0627 (MSSA476)              | EVVLGILGIVLIITGGYLSLNIVQYYDSIGTLMFILLSTVIGAYLFFKSS  | 250 |
| SAB0611 (RF122)                | EVVLGILGIVLIITGGYLSLNIVQYYDSIGTLMFILLSTVIGAYLFFKSS  | 250 |
| Consensus                      | *****                                               |     |
|                                | EVVLGILGIVLIXTGGYLSLNIVQYYDSIGXLMFILLSTVIGAYLFFKSS  |     |
| SACOL0720_                     | VSLVFKMVKKFRKGVISVNDVMFSSSIMYRIKKNAFSLTVMAIISAITVS  | 300 |
| SAUSA300_0648 (USA300-FPR3757) | VSLVFKMVKKFRKGVISVNDVMFSSSIMYRIKKNAFSLTVMAIISAITVS  | 300 |
| SAOUHSC_00668 (NCTC8325)       | VSLVFKMVKKFRKGVISVNDVMFSSSIMYRIKKNAFSLTVMAIISAITVS  | 270 |
| NWMN_0631 (Newman)             | VSLVFKMVKKFRKGVISVNDVMFSSSIMYRIKKNAFSLTVMAIISAITVS  | 255 |
| SaurJH1_0700 (JH1)             | VSLVFKMVKKFRKGVISVNDVMFSSSIMYRIKKNAFSLTVMAIISAITVS  | 255 |
| SaurJH9_0685 (JH9)             | VSLVFKMVKKFRKGVISVNDVMFSSSIMYRIKKNAFSLTVMAIISAITVS  | 255 |
| SAHV_0659 (Mu3)                | VSLVFKMVKKFRKGVISVNDVMFSSSIMYRIKKNAFSLTVMAIISAITVS  | 255 |
| SAV0662 (Mu50)                 | VSLVFKMVKKFRKGVISVNDVMFSSSIMYRIKKNAFSLTVMAIISAITVS  | 300 |
| SA0617 (N315)                  | VSLVFKMVKKFRKGVISVNDVMFSSSIMYRIKKNAFSLTVMAIISAITVS  | 300 |
| MW0624 (MW2)                   | VSLVFKMVKKFRKGVISVNDVMFSSSIMYRIKKNAFSLTVMAIISAITVS  | 300 |
| SAS0627 (MSSA476)              | VSLVFKMVKKFRKGVISVNDVMFSSSIMYRIKKNAFSLTVMAIISAITVS  | 300 |
| SAB0611 (RF122)                | VSLVFKMVKKFRKGVISVNDVMFSSSIMYRIKKNAFSLTVMAIISAITVS  | 300 |
| Consensus                      | *****                                               |     |
|                                | VSLVFKMVKKFRKGVISVNDVMFSSSIMYRIKKNAFSLTVMAIISAITVS  |     |
| SACOL0720_                     | VLCFAAISRASLSSEIKYTAPHDVTIKDQOKANQLASELNNQKIPHFYNY  | 350 |
| SAUSA300_0648 (USA300-FPR3757) | VLCFAAISRASLSSEIKYTAPHDVTIKDQOKANQLASELNNQKIPHFYNY  | 350 |
| SAOUHSC_00668 (NCTC8325)       | VLCFAAISRASLSSEIKYTAPHDVTIKDQOKANQLASELNNQKIPHFYNY  | 320 |
| NWMN_0631 (Newman)             | VLCFAAISRASLSSEIKYTAPHDVTIKDQOKANQLASELNNQKIPHFYNY  | 305 |
| SaurJH1_0700 (JH1)             | VLCFAAISRASLSSEIKYTAPHDVTIKDQOKANQLASELNNQKIPHFYNY  | 305 |
| SaurJH9_0685 (JH9)             | VLCFAAISRASLSSEIKYTAPHDVTIKDQOKANQLASELNNQKIPHFYNY  | 305 |
| SAHV_0659 (Mu3)                | VLCFAAISRASLSSEIKYTAPHDVTIKDQOKANQLASELNNQKIPHFYNY  | 305 |
| SAV0662 (Mu50)                 | VLCFAAISRASLSSEIKYTAPHDVTIKDQOKANQLASELNNQKIPHFYNY  | 350 |
| SA0617 (N315)                  | VLCFAAISRASLSSEIKYTAPHDVTIKDQOKANQLASELNNQKIPHFYNY  | 350 |
| MW0624 (MW2)                   | VLCFAAISRASLSSEIKYTAPHDVTIKDQOKANQLASELNNQKIPHFYNY  | 350 |
| SAS0627 (MSSA476)              | VLCFAAISRASLSSEIKYTAPHDVTIKDQOKANQLASELNNQKIPHFYNY  | 350 |
| SAB0611 (RF122)                | VLCFAAISRASLSSEIKYTAPHDVTIKDQOKANQLASELNNQKIPHFYNY  | 350 |
| Consensus                      | *****                                               |     |
|                                | VLCFAAISRASLSSEIKYTAPHDVTIKDQOKANQLASELNNQKIPHFYNY  |     |
| SACOL0720_                     | KEVIHTKLYKDNLFDDVKAKEPYNVTITSDKYIPNTDLKRGQADLFVAEGS | 400 |
| SAUSA300_0648 (USA300-FPR3757) | KEVIHTKLYKDNLFDDVKAKEPYNVTITSDKYIPNTDLKRGQADLFVAEGS | 400 |
| SAOUHSC_00668 (NCTC8325)       | KEVIHTKLYKDNLFDDVKAKEPYNVTITSDKYIPNTDLKRGQADLFVAEGS | 370 |
| NWMN_0631 (Newman)             | KEVIHTKLYKDNLFDDVKAKEPYNVTITSDKYIPNTDLKRGQADLFVAEGS | 355 |
| SaurJH1_0700 (JH1)             | KEVIHTKLYKDNLFDDVKAKEPYNVTITSDKYIPNTDLKRGQADLFVAEGS | 355 |
| SaurJH9_0685 (JH9)             | KEVIHTKLYKDNLFDDVKAKEPYNVTITSDKYIPNTDLKRGQADLFVAEGS | 355 |
| SAHV_0659 (Mu3)                | KEVIHTKLYKDNLFDDVKAKEPYNVTITSDKYIPNTDLKRGQADLFVAEGS | 355 |
| SAV0662 (Mu50)                 | KEVIHTKLYKDNLFDDVKAKEPYNVTITSDKYIPNTDLKRGQADLFVAEGS | 400 |
| SA0617 (N315)                  | KEVIHTKLYKDNLFDDVKAKEPYNVTITSDKYIPNTDLKRGQADLFVAEGS | 400 |
| MW0624 (MW2)                   | KEVIHTKLYKDNLFDDVKAKEPYNVTITSDKYIPNTDLKRGQADLFVAEGS | 400 |
| SAS0627 (MSSA476)              | KEVIHTKLYKDNLFDDVKAKEPYNVTITSDKYIPNTDLKRGQADLFVAEGS | 400 |
| SAB0611 (RF122)                | KEVIHTKLYKDNLFDDVKAKEPYNVTITSDKYIPNTDLKRGQADLFVAEGS | 400 |
| Consensus                      | *****:*****                                         |     |
|                                | KEVIHTKLYKDNLFDDVKKXPYNVTITSDKYIPXTDLKRGQADLFVAEGS  |     |
| SACOL0720_                     | IKDLVKHKKHGKAIIGTKKHHVNIKLRKDINKIYFMTDVDLGGPTFVLND  | 450 |
| SAUSA300_0648 (USA300-FPR3757) | IKDLVKHKKHGKAIIGTKKHHVNIKLRKDINKIYFMTDVDLGGPTFVLND  | 450 |
| SAOUHSC_00668 (NCTC8325)       | IKDLVKHKKHGKAIIGTKKHHVNIKLRKDINKIYFMTDVDLGGPTFVLND  | 420 |
| NWMN_0631 (Newman)             | IKDLVKHKKHGKAIIGTKKHHVNIKLRKDINKIYFMTDVDLGGPTFVLND  | 405 |
| SaurJH1_0700 (JH1)             | IKDLVKHKKHGKAIIGTKKHHVNIKLRKDINKIYFMTDVDLGGPTFVLND  | 405 |
| SaurJH9_0685 (JH9)             | IKDLVKHKKHGKAIIGTKKHHVNIKLRKDINKIYFMTDVDLGGPTFVLND  | 405 |
| SAHV_0659 (Mu3)                | IKDLVKHKKHGKAIIGTKKHHVNIKLRKDINKIYFMTDVDLGGPTFVLND  | 405 |
| SAV0662 (Mu50)                 | IKDLVKHKKHGKAIIGTKKHHVNIKLRKDINKIYFMTDVDLGGPTFVLND  | 450 |
| SA0617 (N315)                  | IKDLVKHKKHGKAIIGTKKHHVNIKLRKDINKIYFMTDVDLGGPTFVLND  | 450 |
| MW0624 (MW2)                   | IKDLVKHKKHGKAIIGTKKHHVNIKLRKDINKIYFMTDVDLGGPTFVLND  | 450 |
| SAS0627 (MSSA476)              | IKDLVKHKKHGKAIIGTKKHHVNIKLRKDINKIYFMTDVDLGGPTFVLND  | 450 |
| SAB0611 (RF122)                | IKDLVKHKKHGKAIIGTKKHHVNIKLRKDINKIYFMTDVDLGGPTFVLND  | 450 |
| Consensus                      | *****:*****                                         |     |
|                                | IKDLVKHKKHGKAXIIGTKKHHVNIKLRKDINKIYFMTDVDLGGPTFVLND |     |

FIG. 23J

|                                |                                                     |     |
|--------------------------------|-----------------------------------------------------|-----|
| SACOL0720_                     | KDYQEIRKYTKAKHIVSQFGFDLKHKKDALALEKAKNKVDKSIETRSEAI  | 500 |
| SAUSA300_0648 (USA300-FPR3757) | KDYQEIRKYTKAKHIVSQFGFDLKHKKDALALEKAKNKVDKSIETRSEAI  | 500 |
| SAOUHSC_00668 (NCTC8325)       | KDYQEIRKYTKAKHIVSQFGFDLKHKKDALALEKAKNKVDKSIETRSEAI  | 470 |
| NWMN_0631 (Newman)             | KDYQEIRKYTKAKHIVSQFGFDLKHKKDALALEKAKNKVDKSIETRSEAI  | 455 |
| SaurJH1_0700 (JH1)             | KDYQEIRKYTKAKHIVSQFGFDLKHKKDALALEKAKNKVDKSIETRSEAI  | 455 |
| SaurJH9_0685 (JH9)             | KDYQEIRKYTKAKHIVSQFGFDLKHKKDALALEKAKNKVDKSIETRSEAI  | 455 |
| SAHV_0659 (Mu3)                | KDYQEIRKYTKAKHIVSQFGFDLKHKKDALALEKAKNKVDKSIETRSEAI  | 455 |
| SAV0662 (Mu50)                 | KDYQEIRKYTKAKHIVSQFGFDLKHKKDALALEKAKNKVDKSIETRSEAI  | 500 |
| SA0617 (N315)                  | KDYQEIRKYTKAKHIVSQFGFDLKHKKDALALEKAKNKVDKSIETRSEAI  | 500 |
| MW0624 (MW2)                   | KDYQEIRKYTKAKHIVSQFGFDLKHKKDALALEKAKNKVDKSIETRSEAI  | 500 |
| SAS0627 (MSSA476)              | KDYQEIRKYTKAKHIVSQFGFDLKHKKDALALEKAKNKVDKSIETRSEAI  | 500 |
| SAB0611 (RF122)                | KDYQEIRKYTKAKHIVSQFGFDLKHKKDALALEKAKNKVDKSIETRSEAI  | 500 |
|                                | *****.*****.*****                                   |     |
| Consensus                      | KDYQEIRKYTKAKHIVSQFGFDLKHKKDALALEKAXNKVDKSIETRSEAI  |     |
| SACOL0720_                     | SSISSLTGILLFVTSFLGITFLIAVCCIIYIKQIDETEDELNYSILRKL   | 550 |
| SAUSA300_0648 (USA300-FPR3757) | SSISSLTGILLFVTSFLGITFLIAVCCIIYIKQIDETEDELNYSILRKL   | 550 |
| SAOUHSC_00668 (NCTC8325)       | SSISSLTGILLFVTSFLGITFLIAVCCIIYIKQIDETEDELNYSILRKL   | 520 |
| NWMN_0631 (Newman)             | SSISSLTGILLFVTSFLGITFLIAVCCIIYIKQIDETEDELNYSILRKL   | 505 |
| SaurJH1_0700 (JH1)             | SSISSLTGILLFVTSFLGITFLIAVCCIIYIKQIDETEDELNYSILRKL   | 505 |
| SaurJH9_0685 (JH9)             | SSISSLTGILLFVTSFLGITFLIAVCCIIYIKQIDETEDELNYSILRKL   | 505 |
| SAHV_0659 (Mu3)                | SSISSLTGILLFVTSFLGITFLIAVCCIIYIKQIDETEDELNYSILRKL   | 505 |
| SAV0662 (Mu50)                 | SSISSLTGILLFVTSFLGITFLIAVCCIIYIKQIDETEDELNYSILRKL   | 550 |
| SA0617 (N315)                  | SSISSLTGILLFVTSFLGITFLIAVCCIIYIKQIDETEDELNYSILRKL   | 550 |
| MW0624 (MW2)                   | SSISSLTGILLFVTSFLGITFLIAVCCIIYIKQIDETEDELNYSILRKL   | 550 |
| SAS0627 (MSSA476)              | SSISSLTGILLFVTSFLGITFLIAVCCIIYIKQIDETEDELNYSILRKL   | 550 |
| SAB0611 (RF122)                | SSISSLTGILLFVTSFLGITFLIAVCCIIYIKQIDETEDELNYSILRKL   | 550 |
|                                | *****                                               |     |
| Consensus                      | SSISSLTGILLFVTSFLGITFLIAVCCIIYIKQIDETEDELNYSILRKL   |     |
| SACOL0720_                     | GFTQKDMARGLKFKIMFNFGPLPLVIALSHAYFTSLAYMKLMGTTNQIPVF | 600 |
| SAUSA300_0648 (USA300-FPR3757) | GFTQKDMARGLKFKIMFNFGPLPLVIALSHAYFTSLAYMKLMGTTNQIPVF | 600 |
| SAOUHSC_00668 (NCTC8325)       | GFTQKDMARGLKFKIMFNFGPLPLVIALSHAYFTSLAYMKLMGTTNQIPVF | 570 |
| NWMN_0631 (Newman)             | GFTQKDMARGLKFKIMFNFGPLPLVIALSHAYFTSLAYMKLMGTTNQIPVF | 555 |
| SaurJH1_0700 (JH1)             | GFTQKDMARGLKFKIMFNFGPLPLVIALSHAYFTSLAYMKLMGTTNQIPVF | 555 |
| SaurJH9_0685 (JH9)             | GFTQKDMARGLKFKIMFNFGPLPLVIALSHAYFTSLAYMKLMGTTNQIPVF | 555 |
| SAHV_0659 (Mu3)                | GFTQKDMARGLKFKIMFNFGPLPLVIALSHAYFTSLAYMKLMGTTNQIPVF | 555 |
| SAV0662 (Mu50)                 | GFTQKDMARGLKFKIMFNFGPLPLVIALSHAYFTSLAYMKLMGTTNQIPVF | 600 |
| SA0617 (N315)                  | GFTQKDMARGLKFKIMFNFGPLPLVIALSHAYFTSLAYMKLMGTTNQIPVF | 600 |
| MW0624 (MW2)                   | GFTQKDMARGLKFKIMFNFGPLPLVIALSHAYFTSLAYMKLMGTTNQIPVF | 600 |
| SAS0627 (MSSA476)              | GFTQKDMARGLKFKIMFNFGPLPLVIALSHAYFTSLAYMKLMGTTNQIPVF | 600 |
| SAB0611 (RF122)                | GFTQKDMARGLKFKIMFNFGPLPLVIALSHAYFTSLAYMKLMGTTNQIPVF | 600 |
|                                | *****.*****.*****                                   |     |
| Consensus                      | GFTQKDMARGLKFKIMFNFGPLPLVIXLSHAYFTSLAYMKLMGTTNQIPVF |     |
| SACOL0720_                     | IVMGLYICMYAVFAVTAYNHSKRTIRHSI                       | 629 |
| SAUSA300_0648 (USA300-FPR3757) | IVMGLYICMYAVFAVTAYNHSKRTIRHSI                       | 629 |
| SAOUHSC_00668 (NCTC8325)       | IVMGLYICMYAVFAVTAYNHSKRTIRHSI                       | 599 |
| NWMN_0631 (Newman)             | IVMGLYICMYAVFAVTAYNHSKRTIRHSI                       | 584 |
| SaurJH1_0700 (JH1)             | IVMGLYICMYAVFAVTAYNHSKRTIRHSI                       | 584 |
| SaurJH9_0685 (JH9)             | IVMGLYICMYAVFAVTAYNHSKRTIRHSI                       | 584 |
| SAHV_0659 (Mu3)                | IVMGLYICMYAVFAVTAYNHSKRTIRHSI                       | 584 |
| SAV0662 (Mu50)                 | IVMGLYICMYAVFAVTAYNHSKRTIRHSI                       | 629 |
| SA0617 (N315)                  | IVMGLYICMYAVFAVTAYNHSKRTIRHSI                       | 629 |
| MW0624 (MW2)                   | IVMGLYICMYAVFAVTAYNHSKRTIRHSI                       | 629 |
| SAS0627 (MSSA476)              | IVMGLYICMYAVFAVTAYNHSKRTIRHSI                       | 629 |
| SAB0611 (RF122)                | IVMGLYICMYAVFAVTAYNHSKRTIRHSI                       | 629 |
|                                | *****                                               |     |
| Consensus                      | IVMGLYICMYAVFAVTAYNHSKRTIRHSI                       |     |

FIG. 23K

**SACOL0029: Multiple polypeptide sequences alignment: hypothetical epitope shaded**

```

SACOL0029 (COL) -----MLESREQLSVEEYETFFNRFDNQEFDF
MW0028 (MW2) -----MLESREQLSVEEYETFFNRFDNQEFDF
SAUSA300_0029 (USA300-FPR3757) -----MLESREQLSVEEYETFFNRFDNQEFDF
SAHV_0037 (Mu3) CLFSYSGSGAVGEIFSGSIVKGYDKALDKEKHLNMLESREQLSVEEYETFFNRFDNQEFDF
SAV0038 (Mu) CLFSYSGSGAVGEIFSGSIVKGYDKALDKEKHLNMLESREQLSVEEYETFFNRFDNQEFDF
SA0035 CLFSYSGSGAVGEIFSGSIVKGYDKALDKEKHLNMLESREQLSVEEYETFFNRFDNQEFDF
SAB2420 -----GEFYSATLVEGYKDHLDQAAHKALLNNRTEVSVDAYETFFKRFDVDFDE
SHY97-3906 GLFSYSGSGSVGEFYSATLVEGYKDHLDQAAHKALLNNRTEVSVDAYETFFKRFDVDFDE
SACOL2561 -----GEFYSATLVEGYKDHLDQAAHKALLNNRTEVSVDAYETFFKRFDVDFDE
CLJ08-1290 GLFSYSGSGSVGEFYSATLVEGYKDHLDQAAHKALLNNRTEVSVDAYETFFKRFDVDFDE
CLJ08-3 GLFSYSGSGSVGEFYSATLVEGYKDHLDQAAHKALLNNRTEVSVDAYETFFKRFDVDFDE
                                     :*:.* :*: :*: :*: :*: :*:
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXLXXXXXSVXXYETFFXRFDXDXDX

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SACOL0029 (COL) ERELTQDPYSKVYLYSIEDHIRTYKIEK
MW0028 (MW2) ERELTQDPYSKVYLYSIEDHIRTYKIEK
SAUSA300_0029 (USA300-FPR3757) ERELTQDPYSKVYLYSIEDHIRTYKIEK
SAHV_0037 (Mu3) ERELTQDPYSKVYLYSIEDHIRTYKIEK
SAV0038 (Mu) ERELTQDPYSKVYLYSIEDHIRTYKIEK
SA0035 ERELTQDPYSKVYLYSIEDHIRTYKIEK
SAB2420 QQDAVHEDRRIFYLSNIENNVREYHRPE
SHY97-3906 QQDAVHEDRRIFYLSNIENNVREYHRPE
SACOL2561 EQDAVHEDRHIFYLSNIENNVREYHRPE
CLJ08-1290 EQDAVHEDRHIFYLSNIENNVREYHRPE
CLJ08-3 EQDAVHEDRHIFYLSNIENNVREYHRPE
          :*:.* :*: :*: :*: :*: :*:
Consensus XXXXXXXXXXXXXLXXIEXXXRXXXXXX

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**FIG. 24**

# I SACOL1867 : Multiple polynucleotide sequences alignment

```

MW1753 (MW2)                TTATTGTTCAATGTGCTTTTGAATAAAATCTTTGATTGAGGCGTAAAGTATACGGCACC 60
SAS1734 (MSSA476)           TTATTGTTCAATGTGCTTTTGAATAAAATCTTTGATTGAGGCGTAAAGTATACGGCACC 60
SACOL1867 (COL)             TTATTGTTCAATGTGCTTTTGAATAAAATCTTTGATTGAGGCGTAAAGTATACGGCACC 60
SAOUHSC_01939 (NCTC8325)    TTATTGTTCAATGTGCTTTTGAATAAAATCTTTGATTGAGGCGTAAAGTATACGGCACC 60
NMN 1704 (Newman)           TTATTGTTCAATGTGCTTTTGAATAAAATCTTTGATTGAGGCGTAAAGTATACGGCACC 60
SAUSA300_1756 (USA300-FPR3757) TTATTGTTCAATGTGCTTTTGAATAAAATCTTTGATTGAGGCGTAAAGTATACGGCACC 60
SaurJH1_1897 (JH1)         TTATTGTTCAATGTGCTTTTGAATAAAATCTTTGATTGAGGCGTAAAGTATACGGCACC 60
SAHV_1796 (Mu3)            TTATTGTTCAATGTGCTTTTGAATAAAATCTTTGATTGAGGCGTAAAGTATACGGCACC 60
SaurJH9_1862 (JH9)         TTATTGTTCAATGTGCTTTTGAATAAAATCTTTGATTGAGGCGTAAAGTATACGGCACC 60
SAV1811 (Mu50)             TTATTGTTCAATGTGCTTTTGAATAAAATCTTTGATTGAGGCGTAAAGTATACGGCACC 60
SA1629 (N315)              TTATTGTTCAATGTGCTTTTGAATAAAATCTTTGATTGAGGCGTAAAGTATACGGCACC 60
SAB1671c (RF122)           TTATTGTTCAATGTGCTTTTGAATAAAATCTTTGATTGAGGCGTAAAGTATACGGCACC 60
*****

MW1753 (MW2)                ATTATATTCAGAACCAATTTTCCCAATACCGCCATACACCACACCTATGACCTCATTGTT 120
SAS1734 (MSSA476)           ATTATATTCAGAACCAATTTTCCCAATACCGCCATACACCACACCTATGACCTCATTGTT 120
SACOL1867 (COL)             ATTATATTCAGAACCAATTTTCCCAATACCGCCATACACCACACCTATGACCTCATTGTT 120
SAOUHSC_01939 (NCTC8325)    ATTATATTCAGAACCAATTTTCCCAATACCGCCATACACCACACCTATGACCTCATTGTT 120
NMN 1704 (Newman)           ATTATATTCAGAACCAATTTTCCCAATACCGCCATACACCACACCTATGACCTCATTGTT 120
SAUSA300_1756 (USA300-FPR3757) ATTATATTCAGAACCAATTTTCCCAATACCGCCATACACCACACCTATGACCTCATTGTT 120
SaurJH1_1897 (JH1)         ATTATATTCAGAACCAATTTTCCCAATACCGCCATACACCACACCTATGACCTCATTGTT 120
SAHV_1796 (Mu3)            ATTATATTCAGAACCAATTTTCCCAATACCGCCATACACCACACCTATGACCTCATTGTT 120
SaurJH9_1862 (JH9)         ATTATATTCAGAACCAATTTTCCCAATACCGCCATACACCACACCTATGACCTCATTGTT 120
SAV1811 (Mu50)             ATTATATTCAGAACCAATTTTCCCAATACCGCCATACACCACACCTATGACCTCATTGTT 120
SA1629 (N315)              ATTATATTCAGAACCAATTTTCCCAATACCGCCATACACCACACCTATGACCTCATTGTT 120
SAB1671c (RF122)           ATTGTATTCTGATCCGATTTTCCCAATACCGCCATACACAACCTCCACGACTCATTATT 120
***

MW1753 (MW2)                AGAATTTAGAACTGGTGATCCTGAATTCGCCGGTTCATGTATGCATCAAAATTTAAAAAT 180
SAS1734 (MSSA476)           AGAATTTAGAACTGGTGATCCTGAATTCGCCGGTTCATGTATGCATCAAAATTTAAAAAT 180
SACOL1867 (COL)             AGAATTTAGAACTGGTGATCCTGAATTCGCCGGTTCATGTATGCATCAAAATTTAAAAAT 180
SAOUHSC_01939 (NCTC8325)    AGAATTTAGAACTGGTGATCCTGAATTCGCCGGTTCATGTATGCATCAAAATTTAAAAAT 180
NMN 1704 (Newman)           AGAATTTAGAACTGGTGATCCTGAATTCGCCGGTTCATGTATGCATCAAAATTTAAAAAT 180
SAUSA300_1756 (USA300-FPR3757) AGAATTTAGAACTGGTGATCCTGAATTCGCCGGTTCATGTATGCATCAAAATTTAAAAAT 180
SaurJH1_1897 (JH1)         AGAATTTAGAACTGGTGATCCTGAATTCGCCGGTTCATGTATGCATCAAAATTTAAAAAT 180
SAHV_1796 (Mu3)            AGAATTTAGAACTGGTGATCCTGAATTCGCCGGTTCATGTATGCATCAAAATTTAAAAAT 180
SaurJH9_1862 (JH9)         AGAATTTAGAACTGGTGATCCTGAATTCGCCGGTTCATGTATGCATCAAAATTTAAAAAT 180
SAV1811 (Mu50)             AGAATTTAGAACTGGTGATCCTGAATTCGCCGGTTCATGTATGCATCAAAATTTAAAAAT 180
SA1629 (N315)              AGAATTTAGAACTGGTGATCCTGAATTCGCCGGTTCATGTATGCATCAAAATTTAAAAAT 180
SAB1671c (RF122)           TAAATTTAAACTGGGGATCCTGAATTTCCAGGCTCGATATATGCATCAAAATTTAAAAAT 180
*****

MW1753 (MW2)                ATTGTCCTTTGATTCTTTTATAGTTCCCTGTAGATTCAAACTGTTTAAAACTATTTTGAGC 240
SAS1734 (MSSA476)           ATTGTCCTTTGATTCTTTTATAGTTCCCTGTAGATTCAAACTGTTTAAAACTATTTTGAGC 240
SACOL1867 (COL)             ATTGTCCTTTGATTCTTTTATAGTTCCCTGTAGATTCAAACTGTTTAAAACTATTTTGAGC 240
SAOUHSC_01939 (NCTC8325)    ATTGTCCTTTGATTCTTTTATAGTTCCCTGTAGATTCAAACTGTTTAAAACTATTTTGAGC 240
NMN 1704 (Newman)           ATTGTCCTTTGATTCTTTTATAGTTCCCTGTAGATTCAAACTGTTTAAAACTATTTTGAGC 240
SAUSA300_1756 (USA300-FPR3757) ATTGTCCTTTGATTCTTTTATAGTTCCCTGTAGATTCAAACTGTTTAAAACTATTTTGAGC 240
SaurJH1_1897 (JH1)         ATTGTCCTTTGATTCTTTTATAGTTCCCTGTAGATTCAAACTGTTTAAAACTATTTTGAGC 240
SAHV_1796 (Mu3)            ATTGTCCTTTGATTCTTTTATAGTTCCCTGTAGATTCAAACTGTTTAAAACTATTTTGAGC 240
SaurJH9_1862 (JH9)         ATTGTCCTTTGATTCTTTTATAGTTCCCTGTAGATTCAAACTGTTTAAAACTATTTTGAGC 240
SAV1811 (Mu50)             ATTGTCCTTTGATTCTTTTATAGTTCCCTGTAGATTCAAACTGTTTAAAACTATTTTGAGC 240
SA1629 (N315)              ATTGTCCTTTGATTCTTTTATAGTTCCCTGTAGATTCAAACTGTTTAAAACTATTTTGAGC 240
SAB1671c (RF122)           ATTATCTTTAATACCTTTTACAGTTCCAGTTGATTCAAATTTGTTGAATGTATTTTGAGC 240
***

MW1753 (MW2)                AGGTAATGGGTAACCAATAACCTTTAATTTTGTATCAACTTTAGCATCTTTTCGCAAAAT 300
SAS1734 (MSSA476)           AGGTAATGGGTAACCAATAACCTTTAATTTTGTATCAACTTTAGCATCTTTTCGCAAAAT 300
SACOL1867 (COL)             AGGTAATGGGTAACCAATAACCTTTAATTTTGTATCAACTTTAGCATCTTTTCGCAAAAT 300
SAOUHSC_01939 (NCTC8325)    AGGTAATGGGTAACCAATAACCTTTAATTTTGTATCAACTTTAGCATCTTTTCGCAAAAT 300
NMN 1704 (Newman)           AGGTAATGGGTAACCAATAACCTTTAATTTTGTATCAACTTTAGCATCTTTTCGCAAAAT 300
SAUSA300_1756 (USA300-FPR3757) AGGTAATGGGTAACCAATAACCTTTAATTTTGTATCAACTTTAGCATCTTTTCGCAAAAT 300
SaurJH1_1897 (JH1)         AGGTAATGGGTAACCAATAACCTTTAATTTTGTATCAACTTTAGCATCTTTTCGCAAAAT 300
SAHV_1796 (Mu3)            AGGTAATGGGTAACCAATAACCTTTAATTTTGTATCAACTTTAGCATCTTTTCGCAAAAT 300
SaurJH9_1862 (JH9)         AGGTAATGGGTAACCAATAACCTTTAATTTTGTATCAACTTTAGCATCTTTTCGCAAAAT 300
SAV1811 (Mu50)             AGGTAATGGGTAACCAATAACCTTTAATTTTGTATCAACTTTAGCATCTTTTCGCAAAAT 300
SA1629 (N315)              AGGTAATGGGTAACCAATAACCTTTAATTTTGTATCAACTTTAGCATCTTTTCGCAAAAT 300
SAB1671c (RF122)           TGGTAAAGGGTATCCAATAACCTTTAATTTTGTATCAACTTTAGCATCTTTTCGCAAAAT 300
*****

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FIG. 25A

|                                |                                                                 |     |
|--------------------------------|-----------------------------------------------------------------|-----|
| MW1753 (MW2)                   | TAATGCTTGGACATTTTCATTAAAAATTAAAGCCTTTTGGTCCACGTTCAACTGCCTTGTTTC | 360 |
| SAS1734 (MSSA476)              | TAATGCTTGGACATTTTCATTAAAAATTAAAGCCTTTTGGTCCACGTTCAACTGCCTTGTTTC | 360 |
| SACOL1867 (COL)                | GAATGCTTGGACATTTTCATTAAAAATTAAAGCCTTTTGGTCCACGTTCAACTGCCTTGTTTC | 360 |
| SAOUHSC_01939 (NCTC8325)       | GAATGCTTGGACATTTTCATTAAAAATTAAAGCCTTTTGGTCCACGTTCAACTGCCTTGTTTC | 360 |
| NWMN_1704 (Newman)             | TAATGCTTGGACATTTTCATTAAAAATTAAAGCCTTTTGGTCCACGTTCAACTGCCTTGTTTC | 360 |
| SAUSA300_1756 (USA300-FPR3757) | GAATGCTTGGACATTTTCATTAAAAATTAAAGCCTTTTGGTCCACGTTCAACTGCCTTGTTTC | 360 |
| SaurJH1_1897 (JH1)             | GAATGCTTGGACATTTTCATTAAAAATTAAAGCCTTTTGGTCCACGTTCAACTGCCTTGTTTC | 360 |
| SAHV_1796 (Mu3)                | GAATGCTTGGACATTTTCATTAAAAATTAAAGCCTTTTGGTCCACGTTCAACTGCCTTGTTTC | 360 |
| SaurJH9_1862 (JH9)             | GAATGCTTGGACATTTTCATTAAAAATTAAAGCCTTTTGGTCCACGTTCAACTGCCTTGTTTC | 360 |
| SAV1811 (Mu50)                 | GAATGCTTGGACATTTTCATTAAAAATTAAAGCCTTTTGGTCCACGTTCAACTGCCTTGTTTC | 360 |
| SA1629 (N315)                  | GAATGCTTGGACATTTTCATTAAAAATTAAAGCCTTTTGGTCCACGTTCAACTGCCTTGTTTC | 360 |
| SAB1671c (RF122)               | GAATGCTTGGACATTTTCATTAAAAATTATAACCATTTGCTCCACGTTCAACAGCATTTTC   | 360 |
|                                | ***** * * * * *                                                 |     |
| MW1753 (MW2)                   | TTCAATATTTCATGACAGAGATGCTTCATCACCOCGGATAATCAGAAATGCTTTTAATTTT   | 420 |
| SAS1734 (MSSA476)              | TTCAATATTTCATGACAGAGATGCTTCATCACCOCGGATAATCAGAAATGCTTTTAATTTT   | 420 |
| SACOL1867 (COL)                | TTCAATATTTCATGACAGAGATGCTTCATCACCOCGGATAATCAGAAATGCTTTTAATTTT   | 420 |
| SAOUHSC_01939 (NCTC8325)       | TTCAATATTTCATGACAGAGATGCTTCATCACCOCGGATAATCAGAAATGCTTTTAATTTT   | 420 |
| NWMN_1704 (Newman)             | TTCAATATTTCATGACAGAGATGCTTCATCACCOCGGATAATCAGAAATGCTTTTAATTTT   | 420 |
| SAUSA300_1756 (USA300-FPR3757) | TTCAATATTTCATGACAGAGATGCTTCATCACCOCGGATAATCAGAAATGCTTTTAATTTT   | 420 |
| SaurJH1_1897 (JH1)             | TTCAATATTTCATGACAGAGATGCTTCATCACCOCGGATAATCAGAAATGCTTTTAATTTT   | 420 |
| SAHV_1796 (Mu3)                | TTCAATATTTCATGACAGAGATGCTTCATCACCOCGGATAATCAGAAATGCTTTTAATTTT   | 420 |
| SaurJH9_1862 (JH9)             | TTCAATATTTCATGACAGAGATGCTTCATCACCOCGGATAATCAGAAATGCTTTTAATTTT   | 420 |
| SAV1811 (Mu50)                 | TTCAATATTTCATGACAGAGATGCTTCATCACCOCGGATAATCAGAAATGCTTTTAATTTT   | 420 |
| SA1629 (N315)                  | TTCAATATTTCATGACAGAGATGCTTCATCACCOCGGATAATCAGAAATGCTTTTAATTTT   | 420 |
| SAB1671c (RF122)               | TTCAACGTTTCATTACTGATATATCCTCATTACCTGGATAATCAGAAATATTTTAAATTTT   | 420 |
|                                | *****                                                           |     |
| MW1753 (MW2)                   | ATATATACCACCATTTCCTTTGTCACCGTCTGGATGGGCAGTAATTCATCGCCAACTTT     | 480 |
| SAS1734 (MSSA476)              | ATATATACCACCATTTCCTTTGTCACCGTCTGGATGGGCAGTAATTCATCGCCAACTTT     | 480 |
| SACOL1867 (COL)                | ATATATACCACCATTTCCTTTGTCACCGTCTGGATGGGCAGTAATTCATCGCCAACTTT     | 480 |
| SAOUHSC_01939 (NCTC8325)       | ATATATACCACCATTTCCTTTGTCACCGTCTGGATGGGCAGTAATTCATCGCCAACTTT     | 480 |
| NWMN_1704 (Newman)             | ATATATACCACCATTTCCTTTGTCACCGTCTGGATGGGCAGTAATTCATCGCCAACTTT     | 480 |
| SAUSA300_1756 (USA300-FPR3757) | ATATATACCACCATTTCCTTTGTCACCGTCTGGATGGGCAGTAATTCATCGCCAACTTT     | 480 |
| SaurJH1_1897 (JH1)             | ATATATACCACCATTTCCTTTGTCACCGTCTGGATGGGCAGTAATTCATCGCCAACTTT     | 480 |
| SAHV_1796 (Mu3)                | ATATATACCACCATTTCCTTTGTCACCGTCTGGATGGGCAGTAATTCATCGCCAACTTT     | 480 |
| SaurJH9_1862 (JH9)             | ATATATACCACCATTTCCTTTGTCACCGTCTGGATGGGCAGTAATTCATCGCCAACTTT     | 480 |
| SAV1811 (Mu50)                 | ATATATACCACCATTTCCTTTGTCACCGTCTGGATGGGCAGTAATTCATCGCCAACTTT     | 480 |
| SA1629 (N315)                  | ATATATACCACCATTTCCTTTGTCACCGTCTGGATGGGCAGTAATTCATCGCCAACTTT     | 480 |
| SAB1671c (RF122)               | ATAAATCCACCGTTGCCTTTGTCACCATTTGGGTGGGCAGTAATTCATCGCCAACTTT      | 480 |
|                                | *** ** *                                                        |     |
| MW1753 (MW2)                   | ATAATCTTTTGATACATGTTTATTTGGTGATAATTGTATTTTTCCTCAATTACAAAACCTGT  | 540 |
| SAS1734 (MSSA476)              | ATAATCTTTTGATACATGTTTATTTGGTGATAATTGTATTTTTCCTCAATTACAAAACCTGT  | 540 |
| SACOL1867 (COL)                | ATAATCTTTTGATACATGTTTATTTGGTGATAATTGTATTTTTCCTCAATTACAAAACCTGT  | 540 |
| SAOUHSC_01939 (NCTC8325)       | ATAATCTTTTGATACATGTTTATTTGGTGATAATTGTATTTTTCCTCAATTACAAAACCTGT  | 540 |
| NWMN_1704 (Newman)             | ATAATCTTTTGATACATGTTTATTTGGTGATAATTGTATTTTTCCTCAATTACAAAACCTGT  | 540 |
| SAUSA300_1756 (USA300-FPR3757) | ATAATCTTTTGATACATGTTTATTTGGTGATAATTGTATTTTTCCTCAATTACAAAACCTGT  | 540 |
| SaurJH1_1897 (JH1)             | ATAATCTTTTGATACATGTTTATTTGGTGATAATTGTATTTTTCCTCAATTACAAAACCTGT  | 540 |
| SAHV_1796 (Mu3)                | ATAATCTTTTGATACATGTTTATTTGGTGATAATTGTATTTTTCCTCAATTACAAAACCTGT  | 540 |
| SaurJH9_1862 (JH9)             | ATAATCTTTTGATACATGTTTATTTGGTGATAATTGTATTTTTCCTCAATTACAAAACCTGT  | 540 |
| SAV1811 (Mu50)                 | ATAATCTTTTGATACATGTTTATTTGGTGATAATTGTATTTTTCCTCAATTACAAAACCTGT  | 540 |
| SA1629 (N315)                  | ATAATCTTTTGATACATGTTTATTTGGTGATAATTGTATTTTTCCTCAATTACAAAACCTGT  | 540 |
| SAB1671c (RF122)               | ATAGTCCTTTGATACATGTTTATTTGGTGATAATTGTATTTTTCCTCAATTGCAAAAACCTGT | 540 |
|                                | *** ** *                                                        |     |
| MW1753 (MW2)                   | CGCATCTTTAAATGAAACGACGCCATTATATGGAATAATTTGTATCTTTAACTTGCGT      | 600 |
| SAS1734 (MSSA476)              | CGCATCTTTAAATGAAACGACGCCATTATATGGAATAATTTGTATCTTTAACTTGCGT      | 600 |
| SACOL1867 (COL)                | CGCATCTTTAAATGAAACGACGCCATTATATGGAATAATTTGTATCTTTAACTTGCGT      | 600 |
| SAOUHSC_01939 (NCTC8325)       | CGCATCTTTAAATGAAACGACGCCATTATATGGAATAATTTGTATCTTTAACTTGCGT      | 600 |
| NWMN_1704 (Newman)             | CGCATCTTTAAATGAAACGACGCCATTATATGGAATAATTTGTATCTTTAACTTGCGT      | 600 |
| SAUSA300_1756 (USA300-FPR3757) | CGCATCTTTAAATGAAACGACGCCATTATATGGAATAATTTGTATCTTTAACTTGCGT      | 600 |
| SaurJH1_1897 (JH1)             | CGCATCTTTAAATGAAACGACGCCATTATATGGAATAATTTGTATCTTTAACTTGCGT      | 600 |
| SAHV_1796 (Mu3)                | CGCATCTTTAAATGAAACGACGCCATTATATGGAATAATTTGTATCTTTAACTTGCGT      | 600 |
| SaurJH9_1862 (JH9)             | CGCATCTTTAAATGAAACGACGCCATTATATGGAATAATTTGTATCTTTAACTTGCGT      | 600 |
| SAV1811 (Mu50)                 | CGCATCTTTAAATGAAACGACGCCATTATATGGAATAATTTGTATCTTTAACTTGCGT      | 600 |
| SA1629 (N315)                  | CGCATCTTTAAATGAAACGACGCCATTATATGGAATAATTTGTATCTTTAACTTGCGT      | 600 |
| SAB1671c (RF122)               | CGCATCTTTAAATGAAACGACGCCATTATATGGAATAATTTGTATCTTTAACTTGCGT      | 600 |
|                                | *****                                                           |     |
| MW1753 (MW2)                   | AACATTCTCTCTGCATTTCGCTATTGTTGTTGTCCTCTTCAACGACTGCAGCATTATTCC    | 660 |
| SAS1734 (MSSA476)              | AACATTCTCTCTGCATTTCGCTATTGTTGTTGTCCTCTTCAACGACTGCAGCATTATTCC    | 660 |
| SACOL1867 (COL)                | AACATTCTCTCTGCATTTCGCTATTGTTGTTGTCCTCTTCAACGACTGCAGCATTATTCC    | 660 |
| SAOUHSC_01939 (NCTC8325)       | AACATTCTCTCTGCATTTCGCTATTGTTGTTGTCCTCTTCAACGACTGCAGCATTATTCC    | 660 |
| NWMN_1704 (Newman)             | AACATTCTCTCTGCATTTCGCTATTGTTGTTGTCCTCTTCAACGACTGCAGCATTATTCC    | 660 |
| SAUSA300_1756 (USA300-FPR3757) | AACATTCTCTCTGCATTTCGCTATTGTTGTTGTCCTCTTCAACGACTGCAGCATTATTCC    | 660 |
| SaurJH1_1897 (JH1)             | AACATTCTCTCTGCATTTCGCTATTGTTGTTGTCCTCTTCAACGACTGCAGCATTATTCC    | 660 |
| SAHV_1796 (Mu3)                | AACATTCTCTCTGCATTTCGCTATTGTTGTTGTCCTCTTCAACGACTGCAGCATTATTCC    | 660 |
| SaurJH9_1862 (JH9)             | AACATTCTCTCTGCATTTCGCTATTGTTGTTGTCCTCTTCAACGACTGCAGCATTATTCC    | 660 |
| SAV1811 (Mu50)                 | AACATTCTCTCTGCATTTCGCTATTGTTGTTGTCCTCTTCAACGACTGCAGCATTATTCC    | 660 |
| SA1629 (N315)                  | AACATTCTCTCTGCATTTCGCTATTGTTGTTGTCCTCTTCAACGACTGCAGCATTATTCC    | 660 |
| SAB1671c (RF122)               | AACATTCTCTCTGCATTTCGCTATTGTTGTTGTCCTCTTCAACGACTGCAGCATTATTCC    | 660 |
|                                | *****                                                           |     |

FIG. 25B

```

MW1753 (MW2)          AGTTACTGAGGTTAGAATGGCTAATGCTGCCATGCTTTTAATGACTATATTTTATTCAT 720
SAS1734 (MSSA476)     AGTTGCTGAGGTTAGAATGGCTAATGCTGCCATGCTTTTAATGACTATATTTTATTCAT 720
SACOL1867 (COL)       AGTTACTGAGGTTAGAATGGCTAATGCTGCCATGCTTTTAATGACTATATTTTATTCAT 720
SAOUHSC_01939 (NCTC8325) AGTTACTGAGGTTAGAATGGCTAATGCTGCCATGCTTTTAATGACTATATTTTATTCAT 720
NWMN_1704 (Newman)    AGTTACTGAGGTTAGAATGGCTAATGCTGCCATGCTTTTAATGACTATATTTTATTCAT 720
SAUSA300_1756 (USA300-FPR3757) AGTTACTGAGGTTAGAATGGCTAATGCTGCCATGCTTTTAATGACTATATTTTATTCAT 720
SaurJH1_1897 (JH1)    AGTTACTGAGGTTAGAATGGCTAATGCTGCCATGCTTTTAATGACTATATTTTATTCAT 720
SAHV_1796 (Mu3)       AGTTACTGAGGTTAGAATGGCTAATGCTGCCATGCTTTTAATGACTATATTTTATTCAT 720
SaurJH9_1862 (JH9)    AGTTACTGAGGTTAGAATGGCTAATGCTGCCATGCTTTTAATGACTATATTTTATTCAT 720
SAV1811 (Mu50)        AGTTACTGAGGTTAGAATGGCTAATGCTGCCATGCTTTTAATGACTATATTTTATTCAT 720
SA1629 (N315)         AGTTACTGAGGTTAGAATGGCTAATGCTGCCATGCTTTTAATGACTATATTTTATTCAT 720
SAB1671c (RF122)     AGTTACTGAGGTTAGAATGGCTAATGCTGCCATGCTTTTAATGACTATATTTTATTCAT 720
*****
MW1753 (MW2)          -----
SAS1734 (MSSA476)     -----
SACOL1867 (COL)       AAAAGCACATTGAACAATAA 740
SAOUHSC_01939 (NCTC8325) -----
NWMN_1704 (Newman)    -----
SAUSA300_1756 (USA300-FPR3757) -----
SaurJH1_1897 (JH1)    -----
SAHV_1796 (Mu3)       -----
SaurJH9_1862 (JH9)    -----
SAV1811 (Mu50)        -----
SA1629 (N315)         -----
SAB1671c (RF122)     -----

```

FIG. 25C

**II- SACOL1867: Multiple polypeptide sequences alignment: hypothetical epitopes shaded**

```
MW1753 (MW2) MNKNIVIKSMAALAILTSVTGINAAVVEETQQIANAENVTQVKDTNIFPYNGVVSFKDA 60
SAS1734 (MSSA476) MNKNIVIKSMAALAILTSVTGINAAVVEETQQIANAENVTQVKDTNIFPYNGVVSFKDA 60
SACOL1867 (COL) MNKNIVIKSMAALAILTSVTGINAAVVEETQQIANAENVTQVKDTNIFPYNGVVSFKDA 60
SAOUHSC_01939 (NCTC8325) MNKNIVIKSMAALAILTSVTGINAAVVEETQQIANAENVTQVKDTNIFPYNGVVSFKDA 60
NWMN_1704 (Newman) MNKNIVIKSMAALAILTSVTGINAAVVEETQQIANAENVTQVKDTNIFPYNGVVSFKDA 60
SAUSA300_1756 (USA300-FPR3757) MNKNIVIKSMAALAILTSVTGINAAVVEETQQIANAENVTQVKDTNIFPYNGVVSFKDA 60
SaurJH1_1897 (JH1) MNKNIVIKSMAALAILTSVTGINAAVVEETQQIANAENVTQVKDTNIFPYNGVVSFKDA 60
SAHV_1796 (Mu3) MNKNIVIKSMAALAILTSVTGINAAVVEETQQIANAENVTQVKDTNIFPYNGVVSFKDA 60
SaurJH9_1862 (JH9) MNKNIVIKSMAALAILTSVTGINAAVVEETQQIANAENVTQVKDTNIFPYNGVVSFKDA 60
SAV1811 (Mu50) MNKNIVIKSMAALAILTSVTGINAAVVEETQQIANAENVTQVKDTNIFPYNGVVSFKDA 60
SA1629 (N315) MNKNIVIKSMAALAILTSVTGINAAVVEETQQIANAENVTQVKDTNIFPYNGVVSFKDA 60
SAB1671c (RF122) MNKNIVIKSMAALAILTSVTGINAAVDETTQIANAENVTQVKDTNIFPYNGVVSFKDA 60
*****
Consensus MNKNIVIKSMAALAILTSVTGINAAVXETQQIANAENVTQVKDTNIFPYNGVVSFKDA

MW1753 (MW2) TGFVIGKNTIITNKHVSKDYKVGDRITAHFPGDKGNGGIYKIKSISDYPGDEDISVMNIE 120
SAS1734 (MSSA476) TGFVIGKNTIITNKHVSKDYKVGDRITAHFPGDKGNGGIYKIKSISDYPGDEDISVMNIE 120
SACOL1867 (COL) TGFVIGKNTIITNKHVSKDYKVGDRITAHFPGDKGNGGIYKIKSISDYPGDEDISVMNIE 120
SAOUHSC_01939 (NCTC8325) TGFVIGKNTIITNKHVSKDYKVGDRITAHFPGDKGNGGIYKIKSISDYPGDEDISVMNIE 120
NWMN_1704 (Newman) TGFVIGKNTIITNKHVSKDYKVGDRITAHFPGDKGNGGIYKIKSISDYPGDEDISVMNIE 120
SAUSA300_1756 (USA300-FPR3757) TGFVIGKNTIITNKHVSKDYKVGDRITAHFPGDKGNGGIYKIKSISDYPGDEDISVMNIE 120
SaurJH1_1897 (JH1) TGFVIGKNTIITNKHVSKDYKVGDRITAHFPGDKGNGGIYKIKSISDYPGDEDISVMNIE 120
SAHV_1796 (Mu3) TGFVIGKNTIITNKHVSKDYKVGDRITAHFPGDKGNGGIYKIKSISDYPGDEDISVMNIE 120
SaurJH9_1862 (JH9) TGFVIGKNTIITNKHVSKDYKVGDRITAHFPGDKGNGGIYKIKSISDYPGDEDISVMNIE 120
SAV1811 (Mu50) TGFVIGKNTIITNKHVSKDYKVGDRITAHFPGDKGNGGIYKIKSISDYPGDEDISVMNIE 120
SA1629 (N315) TGFVIGKNTIITNKHVSKDYKVGDRITAHFPGDKGNGGIYKIKSISDYPGDEDISVMNIE 120
SAB1671c (RF122) TGFVIGKNTIITNKHVSKDYKVGDRITAHFPGDKGNGGIYKIKSISDYPGDEDISVMNIE 120
*****
Consensus TGFVIGKNTIITNKHVSKDYKVGDRITAHFPGDKGNGGIYKIKSISDYPGDEDISVMNIE

MW1753 (MW2) EQAVERGPKGFENFENVOALNFAKDAKVDKIKVIGYPLPAQNSFKQFESTGTIKRIKDN 180
SAS1734 (MSSA476) EQAVERGPKGFENFENVOALNFAKDAKVDKIKVIGYPLPAQNSFKQFESTGTIKRIKDN 180
SACOL1867 (COL) EQAVERGPKGFENFENVOALNFAKDAKVDKIKVIGYPLPAQNSFKQFESTGTIKRIKDN 180
SAOUHSC_01939 (NCTC8325) EQAVERGPKGFENFENVOALNFAKDAKVDKIKVIGYPLPAQNSFKQFESTGTIKRIKDN 180
NWMN_1704 (Newman) EQAVERGPKGFENFENVOALNFAKDAKVDKIKVIGYPLPAQNSFKQFESTGTIKRIKDN 180
SAUSA300_1756 (USA300-FPR3757) EQAVERGPKGFENFENVOALNFAKDAKVDKIKVIGYPLPAQNSFKQFESTGTIKRIKDN 180
SaurJH1_1897 (JH1) EQAVERGPKGFENFENVOALNFAKDAKVDKIKVIGYPLPAQNSFKQFESTGTIKRIKDN 180
SAHV_1796 (Mu3) EQAVERGPKGFENFENVOALNFAKDAKVDKIKVIGYPLPAQNSFKQFESTGTIKRIKDN 180
SaurJH9_1862 (JH9) EQAVERGPKGFENFENVOALNFAKDAKVDKIKVIGYPLPAQNSFKQFESTGTIKRIKDN 180
SAV1811 (Mu50) EQAVERGPKGFENFENVOALNFAKDAKVDKIKVIGYPLPAQNSFKQFESTGTIKRIKDN 180
SA1629 (N315) EQAVERGPKGFENFENVOALNFAKDAKVDKIKVIGYPLPAQNSFKQFESTGTIKRIKDN 180
SAB1671c (RF122) ENAVERGANGYNFENVOALNFAKDAKVDKIKVIGYPLPAQNTFFQFESTGTIVKSIKDN 180
*****
Consensus EXAVERGXXGXNENFENVOALNFAKDAKVDKIKVIGYPLPAQNXFKQFESTGTIXKIKDN

MW1753 (MW2) ILNFDAYIEPCNSGSPVLNSNNEVIGVYGGIGKIGSEYNGAVYFTPQIKDFIQKHIEQ 239
SAS1734 (MSSA476) ILNFDAYIEPCNSGSPVLNSNNEVIGVYGGIGKIGSEYNGAVYFTPQIKDFIQKHIEQ 239
SACOL1867 (COL) ILNFDAYIEPCNSGSPVLNSNNEVIGVYGGIGKIGSEYNGAVYFTPQIKDFIQKHIEQ 239
SAOUHSC_01939 (NCTC8325) ILNFDAYIEPCNSGSPVLNSNNEVIGVYGGIGKIGSEYNGAVYFTPQIKDFIQKHIEQ 239
NWMN_1704 (Newman) ILNFDAYIEPCNSGSPVLNSNNEVIGVYGGIGKIGSEYNGAVYFTPQIKDFIQKHIEQ 239
SAUSA300_1756 (USA300-FPR3757) ILNFDAYIEPCNSGSPVLNSNNEVIGVYGGIGKIGSEYNGAVYFTPQIKDFIQKHIEQ 239
SaurJH1_1897 (JH1) ILNFDAYIEPCNSGSPVLNSNNEVIGVYGGIGKIGSEYNGAVYFTPQIKDFIQKHIEQ 239
SAHV_1796 (Mu3) ILNFDAYIEPCNSGSPVLNSNNEVIGVYGGIGKIGSEYNGAVYFTPQIKDFIQKHIEQ 239
SaurJH9_1862 (JH9) ILNFDAYIEPCNSGSPVLNSNNEVIGVYGGIGKIGSEYNGAVYFTPQIKDFIQKHIEQ 239
SAV1811 (Mu50) ILNFDAYIEPCNSGSPVLNSNNEVIGVYGGIGKIGSEYNGAVYFTPQIKDFIQKHIEQ 239
SA1629 (N315) ILNFDAYIEPCNSGSPVLNSNNEVIGVYGGIGKIGSEYNGAVYFTPQIKDFIQKHIEQ 239
SAB1671c (RF122) NLNFDAYIEPCNSGSPVLNLNNEVIGVYGGIGKIGSEYNGAVYFTPQIKEFIQKHIEQ 239
*****
Consensus XLNFDAYIEPCNSGSPVLNXXNNEVIGVYGGIGKIGSEYNGAVYFTPQIKXFIQKHIEQ
```

**FIG. 25D**

**I- SACOL1715 (hemB) : Nucleotide sequence (SEQ ID NO: 181)**

atgaaatttgatagacatagaagattgagatcatcagcgacaatgagagatatggtaga  
gagaatcatgtaagaaaagaagatttaatatccaatTTTTGTAGTTGAAAAAGACGAT  
GTGAAAAAGAAATTAAGTCATTGCCAGGTGTATACCAAATCAGTTTGAATTTACTTGAA  
AGTGAATTAAGAAGCTTATGACTTAGGCATACGTGCCATTATGTTTTTCGGTGTCCA  
AACTCAAAGATGATATAGGTACTGGTGCATACATTCACGATGGTGTATTCAACAGGCA  
ACACGTATTGCTAAAAAATGTATGATGACTTATTAATTGTTGCAGACACTTGTTTATGT  
GAATATACTGATCATGGTCATTGTGGCGTGATTGATGACCATACACATGACGTTGACAAT  
GATAAATCATTGCCACTACTTGTTAAAACAGCAATTTCTCAAGTGGAAGCTGGTGTGAT  
ATTATTGCGCCAAGTAATATGATGGATGGTTTTGTGCTGAAATTCGTCGTGGATTAGAT  
GAAGCCGGCTATTACAATATTCCTATAATGAGTTATGGTGTCAAGTATGCATCAAGTTTC  
TTTGGACCTTTTAGAGATGCAGCAGATTCAGCGCCATCATTTGGGGATAGAAAAACGTAT  
CAGATGGACCCTGCTAACCGTTTGAAGCACTTCGTGAATTAGAAAGTGATCTTAAAGAA  
GGGTGCGACATGATGATTGTTAAACCTGCTCTAAGTTATTTAGATATAGTTCGAGATGTT  
AAAAATCATACGAATGTTCCAGTTGTTGCATATAATGTGAGTGGAGAATATAGTATGACT  
AAAGCAGCGGCACAAAATGGTTGGATAGATGAAGAACGTGTCGTTATGGAACAAATGGTT  
TCAATGAAACGTGCAGGTGCTGATATGATTATTACGTATTTTGCAAAGGACATTTGTCGC  
TATTAGATAAATAA

**II- hemB: amino acid sequence (SEQ ID NO: 182)**

MKFDHRRLRSSATMRDMVRENHVRKEDLIYPIFVVEKDDVKKEIKSLPGVYQISLNLE  
SELKEAYDLGIRAIMFFGVPSKDDIGTGAYIHDGVIQQATRIAKKMYDDLIVADTCLC  
EYTDHGHCVIDDHTHDVDNDKSLPLLVKTAISQVEAGADIIAPSNMMDGFVAEIRRGLD  
EAGYYNIPIMSYGVKYASSFFGPFDAADSAPSFGDRKTYQMDPANRLEALRELESDLKE  
GCDMMIVKPALSYLDIVRDVKNHTNVPVAYNVSGEYSMTKAAAQNGWIDEERVVMEQMV  
SMKRAGADMIITYFAKDICYLKD

**FIG. 26**

**>ClfA (NWMN\_0756, newman) (SEQ ID NO: 183)**

ATGAATATGAAGAAAAAGAAAAACACGCAATTCGGAAAAATCGATTGGCGTGGCTTCAGTGCTTGTAGGTACGTTAAT  
CGGTTTTGGACTACTCAGCAGTAAAGAAGCAGATGCAAGTGAAAATAGTGTTACGCAATCTGATAGCGCAAGTAACGAAA  
GCAAAAGTAATGATTCAAGTAGCGTTAGTGCTGCACCTAAAACAGACGACACAAACGTGAGTGATACTAAAACATCGTCA  
AACACTAATAATGGCGAAACGAGTGTGGCGCAAAATCCAGCACAAACAGGAAACGACACAATCATCATCAACAAATGCAAC  
TACGGAAGAAACGCCGGTAACCTGGTGAAGCTACTACTACGACAACGAATCAAGCTAATACACCGGCAACAACTCAATCAA  
GCAATACAAATGCCGAGGAATTAGTGAATCAAACAAGTAATGAAACGACTTTTAATGATACTAATACAGTATCATCTGTA  
AATTCACCTCAAATCTACAAATGCCGAAAAATGTTTCAACAACGCAAGATACTTCAACTGAAGCAACACCTTCAAACAA  
TGAATCAGCTCCACAGAGTACAGATGCAAGTAATAAAGATGTAGTTAATCAAGCGGTTAATACAAGTGCGCCTAGAATGA  
GAGCATTTAGTTTAGCGGCAGTAGCTGCAGATGCACCGGCAGCTGGCACAGATATTACGAATCAGTTGACGAATGTGACA  
GTTGGTATTGACTCTGGTACGACTGTGTATCCGCACCAAGCAGGTTATGTCAAACCTGAATTATGGTTTTTTCAGTGCCTAA  
TTCTGCTGTTAAAGGTGACACATTCAAAATAACTGTACCTAAAGAATTAACCTTAAATGGTGTAACTTCAACTGCTAAAG  
TGCCACCAATTATGGCTGGAGATCAAGTATTGGCAAATGGTGTAAATCGATAGTGATGGTAATGTTATTTATACATTTACA  
GACTATGTAAATACTAAAGATGATGTAAAAGCAACTTTGACCATGCCCGCTTATATTGACCCTGAAAATGTTAAAAAGAC  
AGGTAATGTGACATTGGCTACTGGCATAGGTAGTACAACAGCAAAACAAAACAGTATTAGTAGATTATGAAAAATATGGTA  
AGTTTTATAACTTATCTATTAAAGGTACAATTGACCAAATCGATAAAACAAAATAATACGTATCGTCAGACAAATTTATGTC  
AATCCAAGTGAGATAACGTTATTGCGCCGGTTTTAACAGGTAATTTAAAACCAAATACGGATAGTAATGCATTAATAGA  
TCAGCAAAATACAAGTATTAAAGTATATAAAGTAGATAATGCAGCTGATTTATCTGAAAGTTACTTTGTGAATCCAGAAA  
ACTTTGAGGATGTCACTAATAGTGTGAATATTACATTCCCAAATCCAAATCAATATAAAGTAGAGTTTAAATACGCCCTGAT  
GATCAAATTACAACACCGTATATAGTAGTTGTTAATGGTCATATTGATCCGAATAGCAAAGGTGATTTAGCTTTACGTTT  
AACTTTATATGGGTATAACTCGAATATAATTTGGCGCTCTATGTCTATGGGACAACGAAGTAGCATTTAATAACGGATCAG  
GTTCTGGTGACGGTATCGATAAACCAGTTGTTCTTGAACAACCTGATGAGCCTGGTGAAATTGAACCAATCCAGAGGAT  
TCAGATTCTGACCCAGGTTGAGATTCTGGCAGCGATTCTAATTCAGATAGCGGTTGAGATTCCGGGTAGTGATTCTACATC  
AGATAGTGGTTCAGATTGAGCGAGTGATTGAGATTGAGCAAGTGATTGAGACTGAGCGAGTGATTGAGATTGAGCAAGCG  
ATTCCGACTCAGCGAGCGATTCCGACTCAGACAATGACTCGGATTGAGATAGCGATTCTGACTCAGACAGTGACTCAGAT  
TCCGACAGTGACTCAGATTGAGATAGCGATTCTGACTCAGACAGTGACTCAGATTGAGATAGCGATTGAGATTGAGATAG  
CGATTGAGATTCCGACAGTGATTCCGACTCAGACAGCGATTCTGACTCCGACAGTGATTCCGACTCAGACAGCGATTGAG  
ATTCCGACAGTGATTCCGACTCAGATAGCGATTCCGACTCAGATAGCGACTCAGATTGAGACAGCGATTGAGATTGAGAC  
AGCGATTGAGATTGAGATAGCGATTGAGATTCCGACAGTGACTCAGATTCCGACAGTGACTCAGATTCCGATTGAGATAGCGATT  
AGATTCCGACAGTGACTCAGATTCCGACAGTGACTCAGACTCAGACAGTGATTCCGATTGAGCGAGTGATTCCGATTGAG  
ATAGTGATTCCGACTCCGACAGTGACTCGGATTGAGATAGCGACTCAGACTCGGATAGCGACTCGGATTGAGATAGCGAT  
TCGGACTCAGATAGCGATTGAGAATCAGACAGCGATTGAGAATCAGACAGCGATTGAGATTGAGACAGCGACTCAGACAG  
TGACTCAGATTGAGATAGTGACTCGGATTGAGCGAGTGATTGAGACTCAGGTAGTGACTCGGATTGATCAAGTGATTCCG  
ACTCAGAAAGTGATTCAAATAGCGATTCCGAGTCAGGTTCTAACAATAATGTAGTTCCGCCTAATTCACCTAAAAATGGT  
ACTAATGCTTCTAATAAAAAATGAGGCTAAAGATAGTAAAGAACCATTACCAGATACAGGTTCTGAAGATGAAGCAAATAC  
GTCATAATTTGGGGATTATTAGCATCAATAGGTTTACTACTTTTCAGAAGAAAAAAGAAAAATAAAGATAAGAAAT  
AA

**>ClfA (NWMN\_0756, newman) (SEQ ID NO: 184)**

MNMKKKEKHAIRKKSIGVASVLVGTLLIGFGLSSKEADASENSVTQSDSASNESKSNDSVVSAAPKTDNTNVDTKTSS  
NTNNGETSVAQNPAQQETTQSSSTNATTEETPVTGEATTTTNTQANTPATTQSSNTNAEELVNQTSNETTFNDTNTVSSV  
NSPQNSTNAENVSTTQDSTTEATPSNNESAPQSTDASNKDVVNQAVNTSAPRMRAFSLAAVAADAPAAGTDITNQLTNVT  
VGIDSGTTVYPHQAGYVKLNYGFSVPNSAVKGDTFKITVPKELNLNGVSTAKVPPIMAGDQVLANGVIDSDGNVIYTF  
DYVNTKDDVKATLTMPAYIDPENVKKTGNVTLATGIGSTTANKTVLVDYEKYGKFYNLSIKGTIDQIDKTNNTYRQTIYV  
NPSGDNVIAPVLTGNLKPNTDSNALIDQQNTSIKVYKVDNAADLSESYFVNPNFEDVTNSVNITFPNPNQYKVEFNTPD  
DQITTPYIVVNGHIDPNSKGDALRLSTLYGYSNIIWRSMWDNEVAFNNGSGSGDGIDKPVVPEQPDEPGEIEPIPED  
SDSDPGSDSGSDSNSDSGSDSGSDSTSDSGSDSASDSASDSASDSASDSASDSASDSASDSASDSASDSASDSASDS  
SDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSD  
SDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSD  
SDSDSDSESDSESDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSD  
TNASNKNEAKDSKEPLDPTGSEDEANTSLIWGLLASIGSLLLFRKKENKDKK

**FIG. 27**

## INTERNATIONAL SEARCH REPORT

International application No.

**PCT/CA2017/051253**

## A. CLASSIFICATION OF SUBJECT MATTER

IPC: **C07K 14/31** (2006.01), **A61K 39/085** (2006.01), **A61P 31/04** (2006.01), **A61P 37/04** (2006.01),  
**C07K 19/00** (2006.01), **C12N 1/21** (2006.01) (more IPCs on the last page)

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
 Keywords used across the whole IPC

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Databases: Genomequest

Keywords: Search of SEQ ID NO:55 in Genomequest

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                            | Relevant to claim No.       |
|-----------|---------------------------------------------------------------------------------------------------------------|-----------------------------|
| Y         | CA2758490 (BAGNOLI et al.) 21 October 2010 (21-10-2010)<br>*see the polypeptide set forth in SEQ ID NO :117*  | 1-3, 10-13, 15-21 and 23-36 |
| Y         | CA2792956 (MALOUIN et al.) 22 September 2011 (22-09-2011)<br>*see the polypeptide set forth in SEQ ID NO :78* | 1-3, 10-13, 15-21 and 23-36 |

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

|                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|--------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| *<br>"A"<br>"E"<br>"L"<br>"O"<br>"P" | Special categories of cited documents:<br>document defining the general state of the art which is not considered to be of particular relevance<br>earlier application or patent but published on or after the international filing date<br>document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)<br>document referring to an oral disclosure, use, exhibition or other means<br>document published prior to the international filing date but later than the priority date claimed | "T"<br>"X"<br>"Y"<br>"&" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention<br>document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone<br>document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art<br>document member of the same patent family |
|--------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

Date of the actual completion of the international search  
 15 December 2017 (15-12-2017)

Date of mailing of the international search report  
 21 December 2017 (21-12-2017)

Name and mailing address of the ISA/CA  
 Canadian Intellectual Property Office  
 Place du Portage I, C114 - 1st Floor, Box PCT  
 50 Victoria Street  
 Gatineau, Quebec K1A 0C9  
 Facsimile No.: 819-953-2476

Authorized officer  
  
 Stephanie Etchells (819) 639-7796

## INTERNATIONAL SEARCH REPORT

International application No.

**PCT/CA2017/051253****Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☒ Claim Nos.: 1-36 (partially)  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

See extra sheet

3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

See extra sheet for a description of the groups.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

**Continuation of: Box No. II**

The International Searching Authority has not carried out a search for claim 1, under PCT Article 17(2)(b). The description, claims, and/or drawings fail to comply with the prescribed requirements to such an extent that a meaningful search could not be carried out. Claim 1 so lacks clarity and/or support that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been established for the parts of the application which appear to be clear and supported. The polypeptide of greatest interest, based on the instant description and the examples section, was determined to be the polypeptide set forth in SEQ ID NO:55 and therefore the search was limited to said polypeptide.

**Continuation of: Box No. III**

The claims are directed to a plurality of inventive concepts as follows:

Group A - Claims 1-3, 10-13 and 15-36 (partially) are directed to the peptide set forth in SEQ ID NO:55, its corresponding nucleic acid sequence as set forth in SEQ ID NO:54 and all vectors, host cells, compositions, kits and uses thereof;

Group B - Claims 1-3 and 10-36 (partially) are directed to the polypeptide set forth in SEQ ID NO:57, its corresponding nucleic acid sequence as set forth in SEQ ID NO:56 and all vectors, host cells, compositions, kits and uses thereof; and

Group C - Claims 1-36 (partially) are directed to a multitude of fusion constructs, their corresponding nucleic acid molecules, vectors, host cells, kits, compositions and use thereof. Group C can be subdivided into a multitude of different groups of invention, each based on a different fusion construct or group of related fusion constructs.

An *a priori* analysis has concluded that peptide epitopes from *Staphylococcus aureus* (NCBI reference Sequence NC\_002951.2) are known in the art (see CA2758490). Additionally using said peptide epitopes in a fusion protein comprising a linker between said peptide epitopes is also known (see CA2758490), and therefore there is no common inventive concept linking the claims together. The claims must be limited to one inventive concept as set out in PCT Rule 13. As claim 1 makes reference to an infinite number of polypeptides, each and every polypeptide representing a possible inventive concept the examiner has chosen to limit the search to the polypeptide set forth in SEQ ID NO:55 as this was the peptide of greatest interest in the instant description and examples section of the instant specification. The examiner has not chosen to examine the first fusion protein mentioned since it was not made nor was its amino acid sequence disclosed.

*C12N 15/31* (2006.01), *C12N 15/62* (2006.01), and *C12N 15/63* (2006.01)

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

International application No.  
**PCT/CA2017/051253**

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